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Contents

Über zwei Neue Glykoside der Tabakblätter. (I).

Über die Chemischen Bestandteile des Tabaks.–II. Mitteilung.

Von

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(*Aus dem Agr.-chem, Laboratorium der Univ, Kyushu.*).

(Eingegangen am 10. Nov. 1931).

(A) Tabacinin.

(1) *Isolierung*: — Die Pulver von getrockneten, japanischen Tabakblät tern werden mit Calciumcarbonat gemischt und mit 95 proc. Alkohol warm extrahiert. Der alkoholische Auszug, nach Entfernung des Wachses, welches beim Erkalten sich abscheidet, wird in Vakuum eingedampft, der Rückstand mit Wasser behandelt und filtriert. Die wässerige Lösung wird mit neutralem Bleiazetat gefällt, der Niederschlag mit verdünnter Schwefelsäure zersetzt und stark eingeengt. Die Lösung, nach wiederholter Extraktion mittels Aether, wird erschöpfend mit Essigäther heiss extrahiert und der nach Eindampfen des Essigäthers gewonnene Sirup in einer kleinen Menge Wasser gelöst. Beim langere Stehenlassen scheiden sich hellgelbe, körnige Aggregate ab, die durch Umkrystallization aus verdünntem Alkohol lange, blassgelbe, glänzende Nadeln liefern, welche ich Tabacinin genannt habe. Ausbeute, 0.01～0.03%.

(2) *Eigenschaften*: — In kaltem Wasser oder Alkohol ist Tabacinin schwer, in heissem Alcohol leicht, aber Äther unlöslich. Ferrichlorid gibt grüne Färbung; Bleiazetat verursacht einen gelben Niederschlag. Mit gelber Farbe wird Tabacinin von konzentrierter Schwefelsäure gelöst. Tabacinin begimt beim Erhitzen auf 175°C sich bräunen und zersetzt sich bei 227°C unter Gasentwicklunge.

(3) *Hydrolyse*: — 2 g. Glykosid werden in 200c.c. 5% schwefelsäure-haltigem, verdünntem Alkohol suspendiert, 5 Studen gekocht, dann der Alkohol abdestilliert und Wasser zugefügt. Die ausfallenden, gelben, nadelförmigen Krystalle werden abfiltriert und aus 50 proc. Alkohol, umkrystallisiert.

(4) *Glucuronsäure*:— Die Hydrolyseslösung, nach Entfernung des Aglycon, wird mit Bariumcarbonat neutralisiert, das Filtrat eingedampft und der weisse, amorphe Rückstand in einer kleinen Menge Wasser aufgenommen. Die wässerige Lösung ist Orzin-salzsäure-und Phlorogluzinsalzsäure-reaktion positiv; reduziert Fehlingsche Lösung; durch Schwefelsäure fällt Bariumsulfat aus.

Glucuronsäurephenylosazon: Smp. 198°C. 15.03% N (Ber. 15.05% N)

Leicht löslich in Aceton, beim Verbrennen bleibt keine Asche zurück.

p-Brom-phenylosazonglucuronsaures Barium: Smp. 215°C. Beim Glühen bleibt sich weisse Asche zurück.

(B) Tabacilin.

(1) *Isolierung*: — Das Filtrat vom Bleiniederschlag, der Tabacinin enthält, wird nach Entbleien mit verd. Schwefelsäure, unter vermindertem Druck zu dickem Sirup eingedampft der Rückstand mit Chloroform extrahiert, der durch Abdestillation des Chloroforms erhaltene Sirup wird wiederholt mit grösseren Mengen Petroleumäther behandelt und so die darin lösbaren Substanzen vollständig beseitigt werden. Wenn dieser Sirup in Alkohol gelöst und wieder mit Petroleumäther gefällt wird, so erhält man einen hellbraunen, wachsartigen Glykosid, der von Verfasser als *"Tabacilin" bezeichnet werden ist. Ausbeute, 0.4~0.5%.

(2) *Eigenschaften*: — Tabacilin ist sauer; löst sich leicht in Alkohol und Chloroform; schwerer in Wasser, Äther, Essigäther und Benzen. Durch 2 proc. kaltes Kaliumhydroxyd gibt Tabacilin keine Zucker, aber kochende verdünnter Mineralsäure spaltet es in Glykose, Nicotin und andere Bestandteile.

(3) *Hydrolyse*: — 30 g. Tabacilin wird in 200 c.c. Alcohol gelöst, 200 c.c. 6 proc. Schwefelsäure zugefügt und 10 Studen zum Sieden erhitzt. Die Flüssigkeit wird eingedampft, mit Wasser verdünnt und mit Äther ausgeschüttelt. Wird aus den ätherischen Extrakten der Äther abdestilliert, so erhält man einen sauerreagierenden Rückstand.

(4) *Nicotin*: — Eine Portion der Hydrolyseslösung wird mit Sodalösung schwach alkalisch gemacht und mit Petroleumäther ausgezogen. Die nach Verdampfen der Petroleumätherlösung zurückbleibende Flüssigkeit hat Nicotingeruch; reagiert alkalisch und färbt sich durch p-Dimethylaminobenzaldehyd und Salzsäure rot.

Nicotindipikrat: — Smp. 219°C, 18.23% N (Ber. 18.05% N).

Nicotinchlorplatinat: — Smp. 34.20% Pt (Ber. 34.09% Pt).

Nicotinchloraurat: — 46.64% Au (Ber. 46.79% Au).

Um weiter festzustellen dass, Nicotin keine Verunreinigung, sondern ein Bestandteil des Glykosids ist, wird Tabacilin in verdünnter Schwefelsäure gelöst und wieder mit Chloroform extrahiert. Das aus der Chloroformlösung, wie oben erwähnt, dargestellte Glykosid gibt Nicotin bei Hydrolyse.

(5) *Glykose*: — Der grössere Teil der Hydrolyseslösung, nach Extraktion mittels Äther, wird mit Bariumcarbonat neutralisiert. Das Filtrat wird eingedampft und der hinterbleibende Sirup, nach Entfernung des Nicotins durch Ausschütteln mittels Petroleumäther, mit warmen Alkohol behandelt. Die alkoholische Lösung wird abgedampft, der Rückstand in Wasser aufgeno-

mmen neutralisiert mit Barytwasser und dann Bleiazetat und Bleiessig gefällt. Das Filtrat vom Bleiniederschlag wird mit verdünnter Schwefelsäure zersetzt. Diese wässerige Lösung reagiert auf die Seliwanoffsche Reaktion negativ; reduziert Fehlingsche Lösung; gibt kein schwerlösliches Phenylhydrazon.

Glykosephenylosazon: — Smp. 207°C, 15.57% N (Ber. 15.64% N).

Glykose-p-bromphenylosazon: — Smp. 212°C, Diese Osazone, bei der Mischprobe, zeigen keine Schmelzpunktsdepression.

Zusammenfassung.

(1) Aus den Tabakblättern wurden zwei neue Glykoside isoliert, die Tabacinin und Tabacilin genannt worden sind.

(2) Bei Hydrolyse, liefert Tabacinin Glucuronsäure und ein gelbes Krystall.

(3) Unter den Hydrolysesprodukten des Tabacilins wurden Nicotin und Glykose nachgewiesen.

On the Mineral Constituents of American "Loess", a Soil of Aeolian Deposit.

By

Sojiro Kawase and Yoshijiro Kihara.

(Received December 26, 1931).

There are three regions known in the world, where aeolian soils, known as "Loess", are widely distributed, i.e. Central China, Central Europe and the northern part of the U. S. A. The mineral composition of "Loess" of China and of Europe is already well investigated, but that of the U. S. A. is not yet described in any literature.

On his tour through America in 1923, Kawase, one of the authers, visited the Soil Bureau in the U. S. Department of Agriculture at Washington, and inquired about the composition of American Loess, but he could get no information concerning this question. So he went to Kansas City where the thick deposit of this aeolian soil is abundantly found and he took a few samples of the soil from a railway-cut in the neibourhood of the city. Those samples were brought to Japan and afterwards studied.

The air-dried Loess is pale yellowish brown in color and contains some "Loesskinder".

Powdered between fingers, the particles of the soil were found to be less

than 2 m.m. in diameter. It can be divided into the following two parts by Sikorski's method:

Sandy part (2~0.05 m.m.) 27.81%, Clayey part (< 0.05 m.m.) 72.19%.

Of the sandy part, the greater portion, about 98% of it, consists of particles less than 0.5 m.m. in diameter.

Then we investigated the mineral consituents of the air-dried sample by means of a polarization-microscope, under the kind guidance of Dr. T. Wakimizu, formerly Professor of Geology in the Tokyo Imperial University.

The result is as follows: This soil consists of quartz, feldspar, hornblende, zirkon and magnetite. (Fig. I and II).

In the Figure I, H denotes hornblende; Z, zirkon, and M, magnetite respectively, and the non-colored mineral constituents, such as quartz and feldspar are not denoted any way. Amoung these constituents, mentioned above, quartz occupies the greatest portion and magnetite and zirkon the smallest. Mica and augite could not be found in the sample.

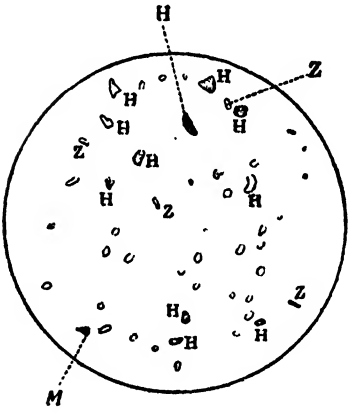


Fig. I

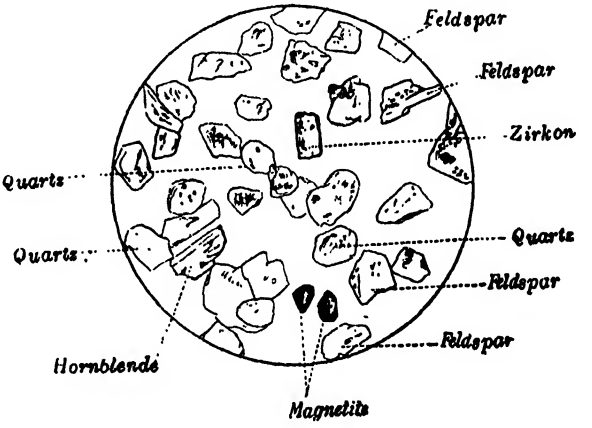


Fig. II

Iodine Contens of Important Agricultural Product in Japan.

By

K. SHIRAHAMA and G. SHIMIZU.

(Received November 26, 1931).

Iodine contents were determined by the method of Leitch and Henderson

somewhat modified by the auther. Results are as follows: —

Sample	Moisture percent	Iodine (γ) per 100 g. of fresh sample.	Iodine (γ) per 100 g. dry matter
Rice	15.40	7.8	9.2
Spring wheat	13.63	22.3	25.7
Winter wheat	14.01	24.5	28.5
Winter rye	13.50	15.0	17.4
Barley (Chevalier)	13.44	19.6	22.6
Barley (Imperial)	12.28	29.3	33.7
Naked barley	12.88	17.0	19.6
Corn	13.92	66.4	77.2
Italian millet	12.95	4.8	5.5
Barnyard millet	11.38	6.9	7.7
Sorghum	14.15	17.5	20.4
Common millet	12.39	14.9	17.0
Soy bean	12.37	47.0	53.6
Azuki bean	14.18	18.6	21.7
Green pea	14.32	64.9	75.7
Cherry	77.86	5.6	25.2
Tomato	93.80	5.1	31.5
Apple (Hōgyōku)	88.02	9.5	86.2
Apple (No. 9)	84.50	3.9	25.3
Spinat	88.67	12.1	106.4
Caboge	91.22	13.5	154.0
Potato	81.04	16.9	89.2
Carrot	83.98	9.8	61.1
Turnip	91.01	6.9	76.2
Onion	91.95	6.5	81.2
Lily	69.46	3.7	12.5
Cucumber	96.35	3.6	99.4
Egg-plant	92.94	5.6	79.0

Our result show that the products in Japan contain 20～80 times as much as European products which were reported by Mayrhofer and Waisitzky, Bleyer, Fellenberg, and McClendon and Remington.

The Protein Requirement of Growing Chicks. (Part I.)

By

Kozo Suzuki and Tadashi Hatano.

(Received November 26, 1931).

In order to find the relative effects of variations in the levels of protein

in the rations upon the growth rate of chicks, upon the age of laying the first egg, upon the body weight at maturity of the female and upon the development of several organs, 180 Single Comb White Leghon day-old chicks, which were hatched in the same incubator, were divided into 3 equal lots, designated as Lot A, B and C, each consisting of 60 chicks.

The following 3 kind of rations were given to each lot separately through all growing period of chicks from day after hatching to 25 weeks of age.

	Lot A	Lot B	Lot C
Rice refuse	10%	10%	10%
Yellow corn	30	30	30
White corn	25	30	35
Rice bran	15	15	15
Fish meal	15	10	5
Salt mixture	5	5	5

Salt mixture consists of 3 parts of bone-meal, 1 part of ground oyster-shell and 1 part of common salt by weight.

The compositions of these rations were as follows: —

	Moisture	Crude protein	Crude fat	Crude fiber	N-free extract	Crude ash
Ration of Lot A	11.62%	18.27%	6.93%	2.39%	51.63%	9.16%
" " B	11.39	15.58	6.61	2.54	55.28	8.60
" " C	11.51	12.85	6.29	2.64	58.62	8.04

The result of all trials in this experiment may be summarized as follows:

All the chicks of two lots, Lot B and C, which received the rations of low protein level, 15.58 and 12.85 percent protein, suffered from heavy diarrhoea and many of them died during the first few weeks of the growing period.

It is necessary to feed a ration relatively high in protein during the first five weeks. A lower protein level than 15.85 percent in the ration is not sufficient for normal growth during the first 10 or 12 weeks of age. It seems that 13 percent protein in the ration may be sufficient for the growing chicks after the first 10 or 12 weeks and until maturity.

There is no effect of the different levels of protein in the rations which were given during the whole growing period of chicks upon the age of laying the first egg.

There is no influence of the different levels of protein in the rations during the growing period upon the development of several organs (liver, kidney, spleen, pancreas, adrenal body, pituitary body, thymus gland and testicle) except for the thyroid gland.

Investigation on Cellulose Decomposition in Soils. (*Continued*)

II. Detection and Estimation of Number of Principal, Aerobic Cellulose Decomposing Bacteria.

By

ARAO ITANO and SATIYO ARAKAWA.

(Received December 2, 1931).

Twenty two samples of air-drid soils, twelve fresh soils and three composts were investigated as to the detection and estimation of number of principal, aerobic cellulose decomposing bacteria by using synthetic cellulose liquid medium after Dubos.

Cytophaga hutchinsoni was found in about 86% soils examined, and in case of the fresh soil, 1,000～100,000 of them were present in 1 g. sample.

In 1 g. of fresh soil of which the reaction was pH (7.0～8.0) 1,000～10,000 of Cellvibrio type bacteria were found but their distribution is limited since they are influenced by the reaction of soil to a great extent.

Although 1,000～110,000 of aerobic cellulose decomposing bacteria were present in 1 g. of fresh soil, they decrease in number to one to ten thousandth when the soil is air-drid. However qualitatively no change was observed.

It was not possible to obtain any information on the anaerobic cellulose decomposing bacteria by the method employed in this investigation.

Further investigation of the bacteria which were isolated is in progress.

On The Natural Pigments of Raw Silk Fibre of the Domestic Cocoon. (Part III).

Melting Point of Cocoon Xanthophyll.

By

MASAMI OKU.

(*From the Chemical Laboratry of Gunze Raw Silk Mfg, Co, Ltd., Ayabe-mati, Kyôto-hu, Japan.*).

(Received December 25, 1931).

In the previous papers I have reported that the main natural yellow pigment of domestic yellow cocoons was xanthophyll, derived biologically from

mulberry leaves upon which silk worms feed and determined its melting point as 175°C (uncorr.).

Recently, xanthophyll was defined by R. Kuhn as the name given to carotinoid group which contains hydroxyl groups and 40 atoms of carbon and he reported that the true melting point of xanthophyll as a chemical entity higher than hitherto usually taken into account.

I have prepared xanthophyll of yellow cocoons and mulberry leaves by the processes given in previous papers once again and purified in according to R. Kuhn by distributing between petroleum ether and 85% methanol, then recrystallizing from mixed solution of methanol and ethyl ether and determined its melting point which showed 193°C (corr.).

As this melting point coincides exactly with that of lutein (leaf xanthophyll), the xanthophyll of yellow cocoons must be lutein and the melting point of xanthophyll formerly reported as 175°C (uncorr.) should be corrected as 193°C (corr.)

Studies on Agar-agar. II.

On the Two Kinds of Hydrato obtained from Hot-water-hydrolysis.

By

E. Takahashi and K. Shirahama.

(Received December 26, 1931).

Two kinds of hydrato were obtained by hot-water-hydrolysis, the one being insoluble in cold water (Hydrato-Kanten-δ) and the other soluble in cold water and precipitated by alcohol from the filtrate of the above (Hydrato-Kanten-λ).

δ seems to be a simple polysaccharide containing almost no ash and is very easily decomposed by dilute mineral acids, however, λ contains much ash and not easily decomposed. Ash composition of λ consisted almost of SO_4 and Ca, and a small amount of Mg, Na and K etc. Through electrodialysis SO_4'' appeares slightly in the anode while $Ca^{\cdot\cdot}$ removed to the cathode very conspicuously and after the electrodialysis the λ solution becomes distinctly acidic. From the above points, the chemical structure of agar is considered as follows: —

$$R\text{–}R'\text{–}O\cdot SO_2\cdot O\text{–}[Me] \qquad [Me]\cdots\cdots Metal$$

In the above formura, the linkage between R and R′ is considered to be rather related to jelly formation, though Samec and Isajevič explained that the jelly formation of agar is due to the linkage between R′ and SO_4.

On the Gluconic Acid Fermentation. (Part III.)

On Bacterium Hoshigaki var glucuronicum I. nov. spec. (Bacterium industrium var Hoshigaki nov. spec.).

By

Teizo Takahashi and Toshinobu Asai.

(Received January 12, 1932).

Bacterium Hoshigaki var glucuronicum I. nov. spec. This bacterium was once described under the name of Bact. industrium var Hoshigaki*. It is shot rod with round end and generally occurs in single but seldom in pairs or often in chains, 1.5～8.0 μ long, 0.5～0.8 μ thick. It is motile and has vigorous tendency to form involution form at room temperature. The optimum temperature for the growth was 25～26°C and the same for the acid production was 24～25°C. The death of this organism was perceived in 5 minutes at 50°C. It forms acid from arabinose, glucose, fractose, galactose, sucrose, maltose, lactose, raffinose, dextrine, starch, inulin, glycol, ethyl alcohol, propyl alcohol, glycerine, mannite, sorbite but not from methyl alcohol. It forms rosy red coloured growth in the medium containing such poly-alcohols as glycol, glycerine, mannite and sorbite.

It makes growth in the solution containing 50% of glucose but not in solution of 55% of the sugar. It could not grow in solution containig 10% of ethyl alcohol. It does not oxidize acetic acid as experienced by Bact. Hoshigaki var rosea. It makes no growth in Beijerinck's solution but inverts cane sugar, so that it could not belong to Bact. aceti Pasteur or Bact. rancens group after Hoyer's system. From Heimann's coccus viz; Bact. gluconicum, this one differs by the property of giving film in fluid media, forming involution forms and acidifying maltose, lactose, starch and inulin. It produces 10.38% of gluconic acid in yeast water containing 10% glucose. This organism produces oxygluconic- and glucuronic acid in presence of Ca-carbonate in the nutrient solution.

* Nippon Nōgei kwagaku Kwaishi, Vol. 6 No. 6, (1930).

On Gluconic Acid Fermentation. (Part IV.)

On Bact. Hoshigaki var glucuronicum II and III nov. spec.

By

Teizo Takahashi and Toshinobu Asai.

(Received January 12, 1932).

In the first communication (Nippon Nōgei Kwagaku Kwaishi Vol. 6. No. 3. March. 1930) on gluconic acid fermentation by authors there was mentioned two acetic bacilli named provisionally Bact. B and C. They were new species in every point of view and their characters are described below.

Bacterium Hoshigaki var glucuronicum II. nov. spec. (*Bact C.*)

It is short rod, 0.8～3.6 μ long and 0.5～1.5 μ thick.

Single or in pairs and motile. It forms viscous film in fluid media, which alters to turbid. The occurrence of involution form is rare. The optimum temperature for the growth is 30～31°C, but the same for the acid formation is 26～28°C.

The death comes at 55°C in 5 minutes. It forms acid from arabinose, glucose, fructose, galactose, sucrose, raffinose, glycerine, mannite, sorbite, ethyl alcohol, propyl alcohol, but not from maltose, lactose, dextrine, starch, inulin, methyl alcohol.

In nutrient solution containing 55～60% of glucose it grows well, the property differing from Bact. Hoshigaki var rosea or Bact. industrium var Hoshigaki (Bact. Hoshigaki var glucuronicum I). It makes growth in solution containing even 12% of ethyl alcohol. It oxidizes acetic acid against to Bact. Hoshigaki var glucuronicum III. It makes no growth in Beijerinck's or Pasteur's solution but could assimilate inorganic nitrogen (ammonium-salt) in Fuhrmann's or Henneberg's second solution. By this property this one differs from Bact. Hoshigaki var rosea and Bact. Hoshigaki var glucuronicum I. But it could not assimilate nitrogen of urea in Henneberg's solution.

It inverts sucrose as Bact. aceti Pasteur group or Bact. xylinum Brown group do. It produces 8.9% of gluconic acid in 10% solution of glucose, but in the solution containing peptone (0.1%) or ammonium phosphate (0.3%) and 10% of glucose the production of gluconic acid increases up to 10%.

Bacterium Hoshigaki var glucuronicum III. nov. spec. (*Bact. B*)

It is short rod of the size of 0.8～2.0 μ × 1.2～4.0 μ, but generally 1.0～1.5 μ × 1.5～3.0 μ. Single, or pairs and motile. It forms viscous film and bacteria ring in fluid media. Involution forms occur rarely, but in glucose yeast

water or Henneberg's solution occur in abundance and even in solid media they occur in seldom.

The optimum temperature for the growth and acid formation is quite same as Bact. Hoshigaki var glucuronicum II. This organism inverts sucrose as Bact. aceti Pasteur group or Bact. xylinum Brown group do. It forms acid from arabinose, glucose, fructose, galactose, sucrose, ethyl alcohol, propyl alcohol, but not from maltose, lactose, raffinose, glycerine, mannite, sorbite, dextrine, starch, inulin and methyl alcohol.

It could not grow in solution containing 50% of glucose, or 5% ethyl alcohol. It could not oxidize acetic acid. It could assimilate inorganic nitrogen (ammonium nitrogen) in Fuhrmann's or Henneberg's IInd solution, although it could not grow in Beijerinck's or Pasteur's solution. By this property this one differs from Bact. Hoshigaki var rosea and Bact. Hoshigaki var glucuronicum I. It could assimilate nitrogen of urea against to Bact. Hoshigaki var glucuronicum II or Bacterium Hoshigaki var rosea. It forms 7.65% gluconic acid in the solution containing 10% of glucose.

Bact. Hoshigaki var glucuronicum II and III also produce oxygluconic- and glucuronic acid in presence of calcium carbonate in the nutrient solution.

Isolation of "Oryzanin" (Antineuritic Vitamin) from Rice-polishings. (First Report.).

By

SATOR OHDAKE.

(Agricultural Chemical Laboratory, Tokyo Imperial University, Faculty of Agriculture, Komaba, Tokyo.)

(Received January 18, 1932).

In 1911, Dr. U. Suzuki[1] isolated an active substance which cures pigeons from polyneuritis, from the alcoholic extract of rice polishings. He proposed the name "Oryzanin" to it and concluded with the view that Oryzanin is a new and hitherto overlooked food factor, essential for animal nutrition beside

(1) U. Suzuki: On an antineuritic substance of rice-bran 1st Rep. J. Chem. Soc. Tokyo. Vol. 32, 1 (1911), 4~17; II. rep. Ibid. Vol. 32, 2 (1911); III. rep. Ibid. Vol. 32, 13 (Apr. 1911); IV. rep. Ibid. Vol. 32, 9 (1911), 874~899; V. rep. Ibid. Vol. 33, 2 (1912), 113~130; VI. rep. Ibid. Vol. 34, 9 (1913); 1123~1125.
U. Suzuki, T. Shimamura and S. Ohdake: Über Oryzanin, ein Wirkamer Bestandteil der Reiskleie und seine physiologische Bedeutung. Bioch. Z. 43 1~2, (1912), 89~153; J. Coll. Agr. Tokyo. Vol. 4, (1913).

proteins, fats, carbohydrates and mineral matters. A numbers of valuable studies[2] on vitamin were reported therafter.

Recently, Jansen and Donath[3] reported that they succeeded in isolating the antineuritic substance in crystalline state from rice polishings. The process used by them is shown in the following schematic table I.

They stated the active substance to be designated by the formula $C_6H_{10}N_2O$ and its hydrochloride $C_6H_{10}N_2O$–HCl, forms needle crystals, melting at 250°C, soluble in water and methyl alcohol but insoluble in cold absolute alcohol and acetone. It gives a precepitate by mercuric chloride, mercuric sulphate and Dragendorf's reagent and also gives intensive Pauly's diazo-reaction. They claimed the protective dose of the hydrochloride to be 0.002 mg. daily for a bondol and 0.03～0.06 mg. for a pigeon.

The present author worked previously on the chemical constituents of yeast extract[4] since 1924 and isolated adenin, cholin, hypoxanthin, leucin, nicotinic acid, thymin, thyrosin, xanthin, adenylthiomethylpentose, methionin, two unknown bases $C_6H_8N_2O$ & $(C_3H_6N_2)_n$ etc. but failed to isolate the active substance in pure state. Furthermore the scanty supply of fresh brewers yeast from the brewery forced the author to apply rice-polishings as the material and the antineuritic preparation "Active Oryzanin" was prepared which cures pigeons from polyneuritis in the dose of 3～4 mg. daily.

Soon after the appearance of Jansen and Donath's publication, the present author tried the same experiment starting from "Active Oryzanin" following the dutch worker's process with minor modifications, and the hydrochloride of antineuritic substance was isolated in crystalline state beside adenin, hypoxanthin, nicotinic acid, cholin, two unknown bases $(C_3H_6N_2)_n$ & $C_8H_{10}NO_2$ etc. The antineuritic substance isolated by the author is the hydrochloride of a new

(2) C. f. Literatures on vitamin: H. C. Sherman & S. L. Smith: The Vitamins; R. Berg: Die Vitamin; C. Funk: The vitamines; W. H. Eddy: The vitamine manual; E. V. McCollum: The newer knowlege of nutrition; U. Suzuki: Chemical studies of vitamin B in Japan, Scientific papers of Institute of physical and chemical research. Vol. 4, 295～302, (1926).

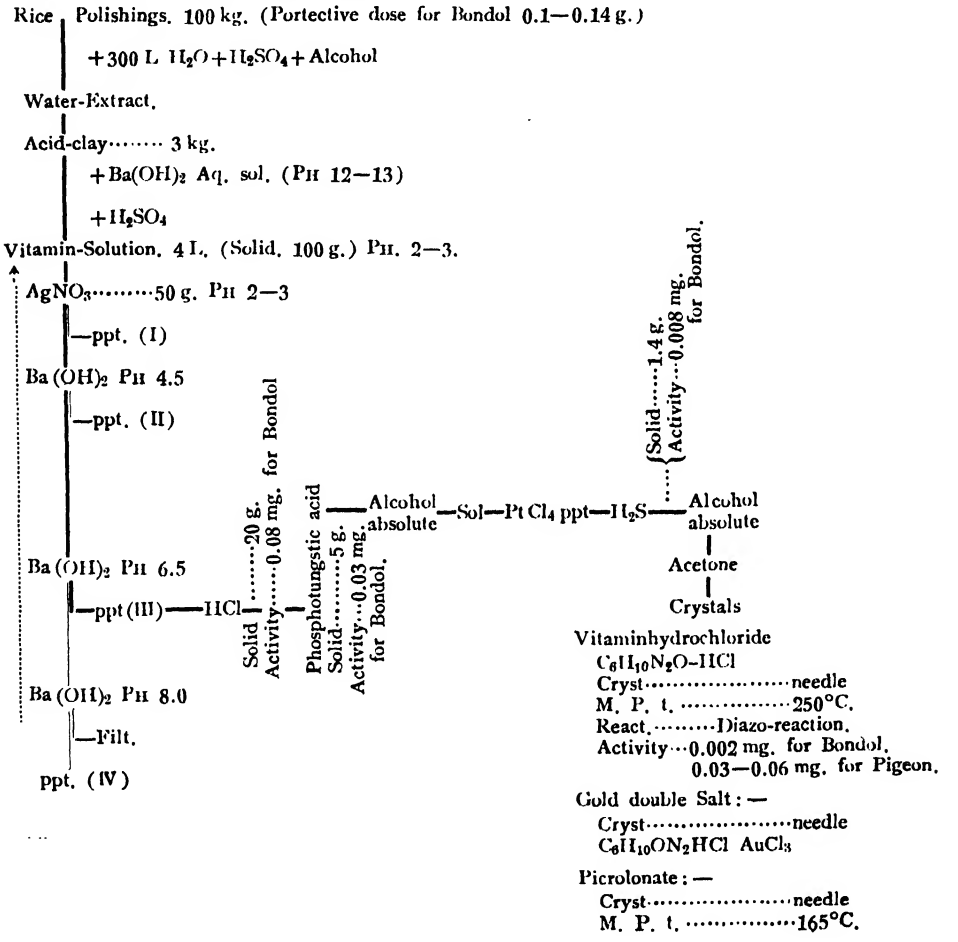
(3) B. C. P. Jansen and W. F. Donath: Isolation of antiberiberi-vitamin; Mededelingen van den Dinst der Volksgezondheid in Ned-indie, Anno, (1927), Part 1 (1927).

(4) S. Ohdake: On the Chemical constituents of yeast-extract. J. Agr. Chem. Soc. Japan. Vol. 3 7～8 (1927) 98～121; II rep. Ibid. Vol. 7 10 (1931), 859～878.
U. Suzuki, S. Ohdake and T. Mori: On the occurrence of a new sulphur compound in yeast. J. Agr. Chem. Soc. Japan. Vol. 1 2 (1924), 127～136; Bioch. Zeit. B 151 II 3～6 (1924), 278～289.
S. Ohdake: On the occurrence of a sulphur containing aminoacid in yeast, J. Agr. Chem. Soc. Japan. Vol. 1 8 (1925), 601～609; Bioch. Zeit. B. 161 II 4～6 (1925), 446～455.
S. Ohdake: On the distribution of a new thioamino acid, Jour. Agr. Chem. Soc. Japan. Vol. 2 10 (1926).

sulphur compound having the formula $C_{12}H_{16}N_4SO_2$. It crystallised in long monoclinic plates melting at 250°C (uncorr.) and its curative dose for a pigeon and a white rat was 0.01–0.02 mg. daily.

Table I. Isolation of Antiberiberi-Vitamin.

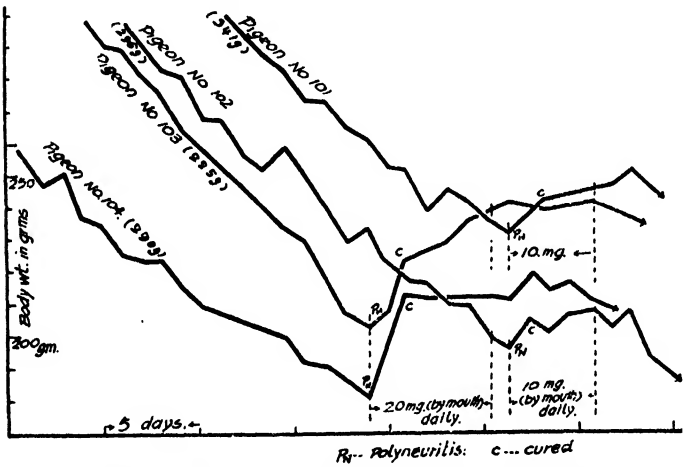
(by Dr. B. C. P. Jansen & W. F. Donath. Medical Laboratory, Weltevreden, Java, Dutch-east-Indies. (1927).



Experimentals.

Preparation of "Active Oryzanin": — Each 100 kg. of fresh rice-polishings were macerated with 800 litres of 0.3% sulphuric acid. After continual stirring for about 3 hours, the mixture was centrifuged and filtered. To the filtrate 5～7 kg. of finely powdered acid-clay was added and stirred for a few hour. The acid-clay adsorbes the greater part of the active substance and subsides

to the bottom, the supernatant fluid was syphoned off. After centrifuging, the sediment was collected and washed with water acidurated by sulphuric acid. To liberate the active substance again, the activated clay was then extracted with 150 litres of 0.5% baryta water and filtered. The second extraction was repeated with 100 litres in the same manner. The united filtrate, freed from an excess of baryta by sulphuric acid, was concentrated in vacuum to a small volume and the requisite quantity of strong alcohol was added to make the alcoholic content of the solution 80% by volume. A voluminous precipitate which consists of proteins and other impurities, was settled and filtered off. When the alcoholic filtrate concentrated to a syrupy consistence containing about 30% of water, the antineuritic concentrate so-colled "Oryzanin-extract" was obtained. The yield was about 250 g. and its antineuritic activity was in daily doses of 12 mg. for pigeons (Chart. 1), therefore about 83% of the active substance contained previously in the original rice-polishings was carried to this concentrate.



Pn-- polyneuritis: c--- cured

Chart. 1--Pigeons on polished rice. & Oryzanin-Extract.

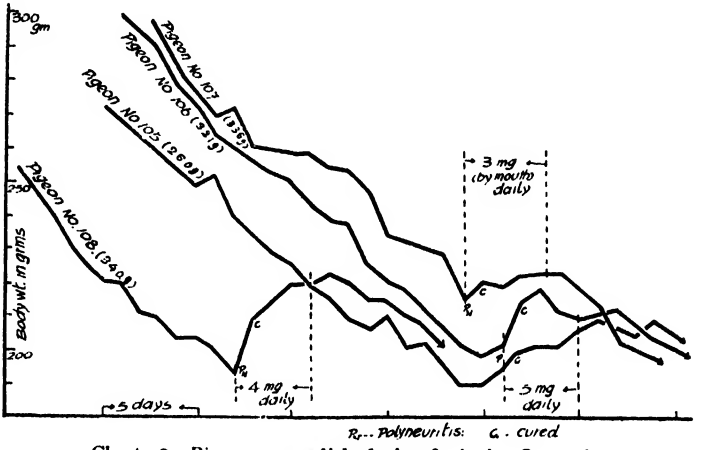
For further concentration 250 g. of "Oryzanin extract" was now dissolved in 2 litres of water and added with the requisite quantity of sulphuric acid to make 5% to the solution, then a 50% aqeous solution of phosphotungstic acid was added. The precipitate which was collected by suction after standing 24 hours, decomposed with baryta in the usual manner. The filtrate freed from an excess of baryta by sulphuric acid, evaporated in vacuum to about 250 g. and 2 litres of 95% alcohol were added to make the alcoholic content of the solution 85% by volume, whereby a voluminous precipitate separated out which were filtered off. The alcoholic filtrate was evaporated in vacuum to dryness. In this way, a light brown mass so-colled "Active Oryzanin" was obtained. The yield was about 33 g. i.e. adequate to 0.03~0.04% of the original rice polishings.

Biological experiment with this substance resulted as follow : —

(1) When pigeons suffering from polyneuritis by exclusive feeding on

polished rice, were supplemented daily with 4mg. of the preparation, they were cured perfectly in a day, improving the symptoms in 4～5 hours. (Chart. 2).

(2) Pigenos, when fed on the synthetical diet consisting of 71% purified starch[5], 15% extracted meat (so-called meat-protein)[6], 10% butter[7], 4% McCollum salts-mixture[8] and administered with 4 mg. of active oryzanin daily, remained entirely health for 50 days against polyneuritis, while symptoms developed after a week by the removal of the supplement. (Chart. 3).



Chert. 2—Pigeons on polished rice & Active-Oryzanin.

(3) Young rats, about 45 g. in weight when developed the symptoms of neuritis by feeding on the synthetical diet, consisting of 70% purified starch, 15% meat-protein, 10% butter, 5% McCollum's salts mixture and with the

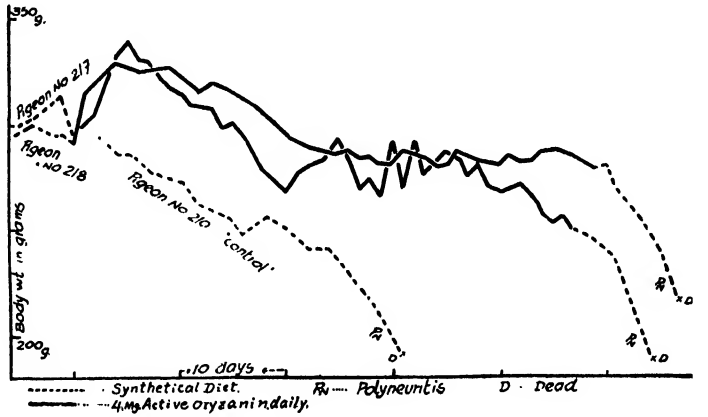


Chart. 3—Pigeons on synthetical diet. & Active Oryzanin.

(5) Purified starch is prepared by extracting "Starch J. P. C." with 80～90% alcohol repeatedly to make free from vitamins and then with ether.

(6) Milled fresh meat is extracted by boiling with enough quantity of water repeatedly and with 80～90% alcohol, then with ether. Extracted meat, prepared by this way, is free from B_1. but still contains the substance which promotes rats-growth when supplemented by antineuritic vitamin.

(7) Butter is purified to make free from B_1. by shaking with warm-water repeatedly and oily layer is collected after centrifuging.

(8) [McCollum's salts mixture. No.185], E. V. McCollum, N. Simonds & W. Pitg: Journ. Biol. Chem. **29** (1917), 521. (Comp. NaCl 0.173··$MgSO_4$ 0.266···$NaH_2PO_4+H_2O$ 0.347 ··K_2HPO_4 0.954···$CaH_4(PO_4)_2+H_2O$ 0.540 ··Fe-Citrate 0.118 ··Ca-Lactate 1.300.)

additon of 3 drops of Codliver oil daily, were cured quickly and grew normally by the supplement of 4 mg. daily. (Chart 4.)

(4) Young rats, fed on the same diet as above, supplemented with 4mg. of active oryzanin daily from the begining of the test, were protected from neuritis and grew perfect healthy. (Chart. 5).

(5) Its pigeon-curative day-dose[9] resulted to be of the order of 0.75 mg;

Pigeon No.	Dose, injected mg.	Days, protected	Day-dose, average mg.
302	5.0	6	0.75
303	5.0	8	
304	5.0	7	
304	5.0	6	

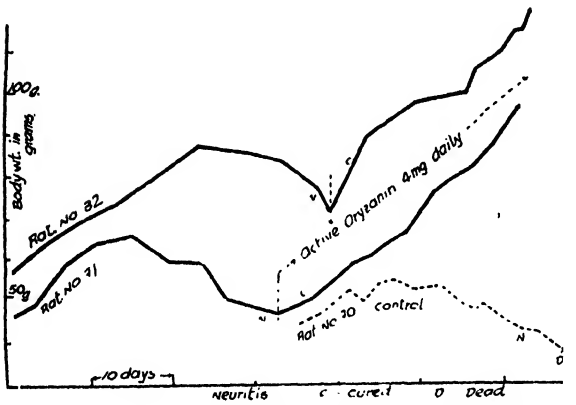


Chart. 4—Rats on synthetical diet, & Active Oryzanin.

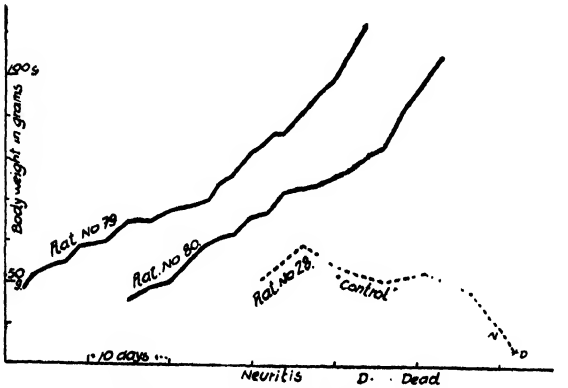


Chart. 5—Rats on synthetical diet, & Active Oryzanin.

From above results, it is ascertained, its anti-neuritic activity to be in daily doses of 4 mg. for pigeons as well as for white rats and its pigeon curative day-dose to be of the order of 0.75 mg.

(6) White rats, fed on the synthetical diet[10] which consisted of 60% purified starch, 20% purified casein, 15% peanuts oil (in place of cottou seed oil), 5% McCollum salts mixture and with the addition of 3 drops of cod-liver oil daily, exhibited the symptoms of neuritis and died after 4 weeks decreasing body weight. (Chart. 6~A).

Rats, fed on the same diet supplemented with 4 mg. of active oryzanin daily, was protected from neuritis but the

(9) Kinnersly and Pelters: Biochem. J., 19 (1925), 820.
(10) Chick and Roscoe: Bioch. J., 22 (1928), 790.

symptoms of pellagra appeared after 5 weeks without promoting growth. (Chart. 6～B).

(7) White rats fed on the same diet as above, supplemented with 0.4 g. of autoclaved yeast daily, exhibited the symptoms of neuritis after 4 weeks, but rats gew normally againt neuritis and pellagra when supplemented with both 4 mg. of active oryzanin and 0.4g autoclaved yeast simultaneously. (Chart. 7).

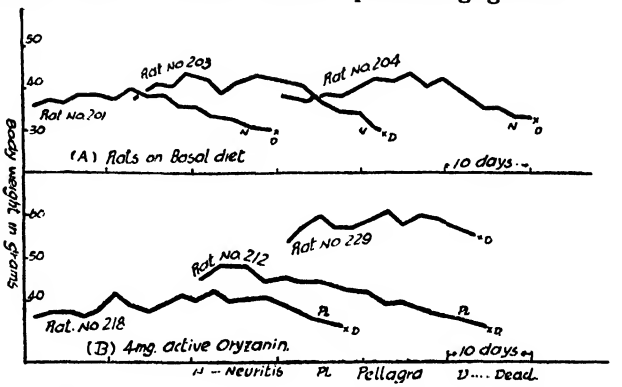


Chart. 6—Rats on synthetical diet. & Active Oryzanin.

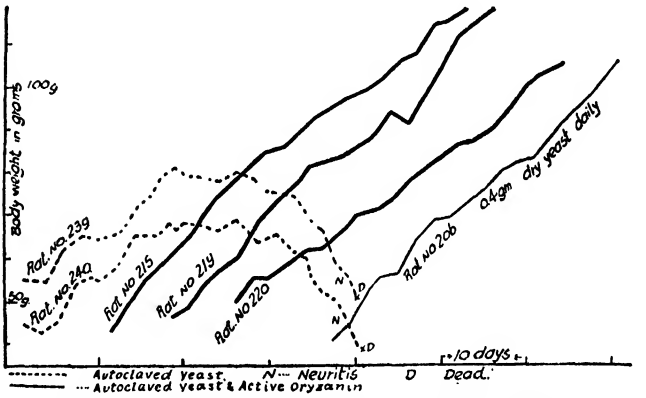


Chart. 7—Rats on synthetical diet, & autoclaved yeast.

For the isolation of antineuritic substance and the allied compounds, active oryzanin was fractionated into five fractions by silver nitrate and baryta. The following account represents a standardised treatment of a batch or 100 g. of "active oryzanin" and five batches were brought together for a lot.

Fractionation by silver nitrate and baryta :— 100 g. of "active oryzanin" were dissolved in 4 litres of water and added with about 15 g. of sulphuric acid to bring the Ph. to 2.5～2.6. A small quantity of resinous substance, thereby separated out, was filtered off. The clear filtrate, brought into a hard-glass beacker of about 10 litres contents, was stirred continuously by means of electric driving and then added with 200 c.c. of 50% aqueous solution of silver nitrate that showed a slight excess. The precipipitate by silver nitrate, allowed to settle for 2～3 hours, filtered by suction and collected on a porous-plate after washing with about 300 c.c. of water. About 25 g. of a dark colored precipitate were obtained which showed to be inactive.....This is called "*Fraction I*".

The filtrate from the "Fraction I" was then treated gradually by constant stirring with the requisite quantity of 4% baryta water to bring the PH to 4.5 and allowed to settle for 2~3 hours. The precipitate which contained chiefly nicotinic acid, was filtered by suction. About 30 g. of a dark gray precipitate were obtained.....*"Fraction II"*.

The filtrate from the "Fraction II" contains the active substance, and its PH was brought to 6.8 by further addition of requisite quantity of baryta water with constant stirring. The dark brown precipitate which carried greater parts of the active substance, was filtered by suction after standing over night. The yield was about 50 g. in average,.....*"Fraction III"*.

More baryta water was then added to the filtrate from the "Fraction III" in the same manner as to the previous fraction to bring the PH to 9.0, and the precipitate formed thus, was filtered after settling down for a few hours. Absut 50 g. of a dark brown precipitate were obtained which contained still a small quantity of active substance.....*"Fraction IV"*.

The filtrate was then treated with hydrochloric acid and sulphuric acid to remove an excess of silver and baryta. The acidity of the solution, having been measured by titration, was brought 5% of sulphuric acid by adding a requisite quantity of sulphuric acid to the filtrate, then a 50% aqueous solution of phosphotungstic acid was added. After standing for about 24 hours, the precipitate was filtered by suction and washed with 5% sulphuric acid. About 190 g. of light gray precipitate were collected.....*"Fretion V"*.

Fraction. I.

The silver precipitate (116 g. from Lot. 1,.....142 g. from Lot. 2.) obtained at PH. 2.5, was suspended in water, and decomposed by hydrochloric acid. The light brown filtrate was treated with a requisite quantity of sulphuric acid to make 5% acidity and then with a 50% aqueous solution of phosphotungstic acid. The precipitate was filtered by suction after standing 24 hours, dissolved in diluted acetone and decomposed by the addition of baryta water. The filtrate from barium phosphotungstate, freed from an excess of

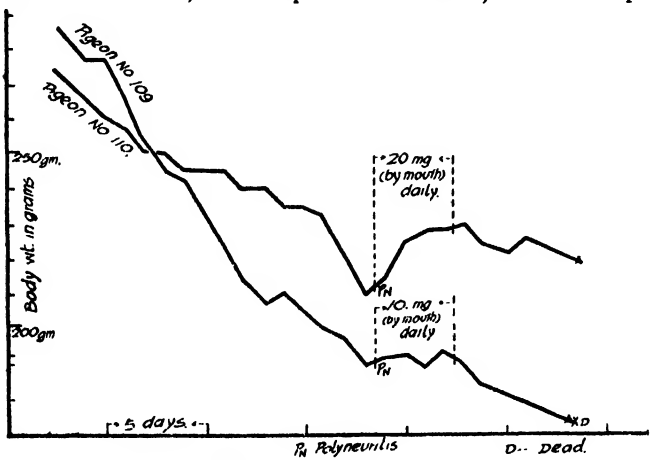


Chart. 8—Pigeons on polished rice. Fraction I.

bayta with sulphuric acid, were concentrated to a small volume. These each contained 21.7 g. (Lot. 1) and 22.5 g. (Lot. 2) of total solids which showed a negligible activity for pigeons. (Chart 8).

On further concentration, white micro-crystalls were separated out.

Yield: 32 g. from 1 kg. of active oryzanin.

(1) *Adenin* $C_5H_5N_5$: — Recrystallised from hot water yield: 25 g. in pure state. White short needles, sparingly soluble in water, gives Kossel's adenin reaction. Its picrate crystallised in characteristic long yellow needles, sparingly soluble in water.

No.	Subst mg.	CO_2 mg.	H_2O mg.	C%	H%	N%
(1)	4.211	6.843	1.416	44.35	3.74	—
(2)	4.838	7.855	1.826	44.28	4.17	—
(3)	1.814	0.802 c.c. N (15°C 757 mm.)			—	52.01
(4)	2.248	0.982 c.c. N (17°C 760 mm.)			—	51.63
(5)	1.902	0.836 c.c. N (16°C 759 mm.)			—	51.87
Calc. for $C_5H_5N_5$				44.44	3.71	51.85

Analysis of the picrate:

No.	Subst mg.	Vol. of N. cc	Temp. C.	Press. mm	N%
(1)	3.257	0.835	15	759	30.37
(2)	3.253	0.845	16	759	30.65
Calc. for $C_5H_5N_5$–$C_6H_3N_3O_7$					30.79

(2) *Adenin–Hypoxanthin*: — The filtrate from adenin, gave on evaporation, 9 g. of fine crystals which were recrystallised from hot water. Yield: 5.3 g. White needles, decomposed at 320°C (uncorr.). It gives Kossel's adenin reaction while Weidel's, xanthin and diazo-reactions are all negative, however, unlike adenin it is easier soluble in water.

No.	Subst mg.	CO_2 mg.	H_2O mg.	C%	H%	N%
(1)	7.040	11.434	2.417	44.29	3.81	—
(2)	2.216	0.862 c.c. N (14°C 764 mm.)			—	46.55
(3)	2.010	0.857 c.c. N (15°C 766 mm.)			—	46.46
Calc. for. $C_5H_5N_5$–$C_5H_4N_4O$				44.28	3.32	46.50

For the purpose of separating adenin from hypoxanthin, it was converted into picrate and subjected to fractional crystallisation, whereby adenin picrate separated out first from the aqueous solution, forming a long yellow needles.

Adenin picrate:

No.	Subst mg.	Vol. of N. cc	Temp. C	Press. mm.	N%
(1)	3.144	0.818	17°	764	30.81
(2)	3.030	0.784	17°	764	30.63
Calc. for $C_5H_5N_5$–$C_6H_3N_3O_7$					30.79

Hypoxanthin picrate: The filtrate of adenin picrate when evaporated to a small volume, separated out the crystals of hypoxanthin picrate forming light yellow plates, melting at 245°C. (uncorr.).

No.	Subst mg.	Vol. of N. cc	Temp. C	Press. mm.	N%
(1)	3.108	0.706	17°	758	26.66
Calc. for $C_5H_4N_4O$–$C_6H_3N_3O_7$					26.85

(3) *Adenin picrate*: — The filtrate of adenin hypoxanthin was evaporated to a small volume and added with picric acid. Upon standing, adenin picrate separated out which was recrystallised from hot water. Yield 1.5 g. Long yellow needles, sparingly soluble in water.

No.	Subst mg.	Vol. of N. cc	Temp. C	Press. mm.	N%
(1)	1.588	0.414	18.5	760	30.54
(2)	1.764	0.438	20.5	760	30.61
Calc. for $C_5H_5N_5$–$C_6H_3N_3O_7$					30.79

(4) *Hypoxanthin picrate*: — The filtrate of adenin picrate when concentrated further, separated out another picrates which were collected and recrystallised from dilute alcohol. Yield: 0.7 g. M. p. 246°C. (uncorr.).

No.	Subst mg.	Vol. of N. cc	Temp. C	Press. mm.	N%
(1)	5.027	1.156	20°	752	26.60
(2)	3.824	0.872	17°	760	26.87
Calc. for $C_5H_4N_4O$–$C_6H_3N_3O_7$					26.85

The above results agrees fairly with hyproxanthin picrate.

Fraction II.

The silver precipitate (403 g. from Lot 1,..... .....360 g. from Lot 2), obtained at the P_H 2.5~4.5, was suspended in water and decomposed by adding an excess of hydrochloric acid. The filtrate from silver chloride was added with the requisite quantity of sulphuric acid to make the acidity of the solution 5 % H_2SO_4, and precipitated with phosphotungstic acid. The precipitate dissolved in diluted acetone, was decomposed with baryta water and

the filtrate was concentrated in vacuum after removing the excess of baryta with sulphuric acid. It contained 96.7 g. of total solid but showed to be inactive. (Chart. 9).

The solution, when evaporated further and kept in a cold place, separated out 67.8 g. of nicotinic acid in needles.

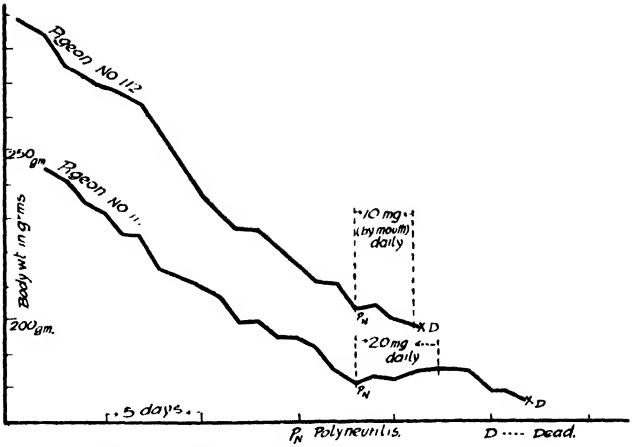


Chart 9—Pigeons on polished rice. Fraction II.

(5) *Nicotinic acid*:— Recrystallised from hot water. Yield: 53.1 g. from 1 kg. of active oryzanin (Lot. 1 & 2). White needes soluble in water and alcohol, melting at 235°C. (uncorr.). Kossel's adenin-reaction as well as Pawly's diazo-reaction were all negative.

No.	Subst. mg.	CO_2 mg.	H_2O mg.	C%	H%	N%
1 (Lot No. 1)	4.928	10.275	1.948	58.06	4.39	—
2 "	4.855	10.388	1.893	58.36	4.34	—
3 (Lot No. 2)	4.337	9.019	1.586	58.60	4.06	—
4 "	4.992	10.720	2.011	58.60	4.48	—
5 (Lot No. 1)	5.523	0.5341 c.c. N (15°C 770 mm.)		—		11.01
6 "	3.754	0.346 c.c. N (15°C 772 mm.)		—		11.10
7 "	5.969	0.600 c.c. N (20°C 750 mm.)		—		11.55
8 "	4.183	0.409 c.c. N (20°C 760 mm.)		—		11.38
Calc. for. $C_6H_5NO_2$				58.54	4.07	11.38

Picrate of nicotinic acid: Light yellow plates, m. p. 219° (uncorr.).

No.	Subst. mg.	Vol. of N. cc	Temp. C	Press. mm.	N%
(1)	3.701	0.510	18°	760	16.16
(2)	3.069	0.416	18°	760	16.09
Calc. for. $C_6H_5NO_2$–$C_6H_3N_3O_7$					15.91

Adenin picrate:— The filtrate from the above crystals when added with picric acid, separated out adenin picrat which were filtered and recrystallised from hot water. Yield 1.3 g.

No.	Subst. mg.	Vol. of N. cc	Temp. C	Press. mm.	N%
(1)	2.625	0.689	19°	763	30.76
(2)	2.119	0.548	18°	763	30.48
Calc. for $C_5H_5N_5$–$C_6H_3N_3O_7$					30.79

(8) *Picrate of nicotinic acid*:— The filtrate of the above picrate, by concentrating further, separated out another picrate which were collected and recrystallised from hot dilute alcohol. Yield: 21.3 g. Light yellow plates, m. p. 219°C (uncorr.).

No.	Subst. mg.	CO_2 mg.	H_2O mg.	C%	H%	N%
(1) Lot No. 1	5.792	8.724	1.143	41.08	2.19	——
(2) Lot No. 2	5.607	8.383	1.137	40.78	2.25	——
(3) Lot No. 1	5.526	0.792 c.c. N (14°C 767 mm.)			——	15.77
(4) Lot No. 1	4.503	0.593 c.c. N (15°C 768 mm.)			——	15.77
(5) Lot No. 2	4.166	0.550 c.c. N (15°C 761 mm.)			——	15.68
(6) Lot No. 2	4.439	0.594 c.c. N (15°C 762 mm.)			——	15.59
Calc. for $C_6H_5NO_2$-$C_6H_3N_3O_7$				41.19	2.27	15.91

Fraction III.

The silver precipitate obtained at the PH 6.8 was decomposed by triturating with an excess of hydrochloric acid. On filtration, a clear light brown solution was obtained which was diluted to 2 litres. The acidity of the solution having been measured by titration, enough sulphuric acid was added to make it 5% acidity and then a 50% aqueous solution of phosphotungstic acid was added. Usually about 50 g. of phosphotungstic acid were required. The precipitate, after standing for 24 hours, was filtered by suction, washed with 5% sulphuric acid, dissolved in diluted acetone and filtered from a very small amount of undissolved residue. The clear dark brown filtrate was poured gradually into about 2 litres of 5% sulphuric acid with constant stirring. A voluminous precipitate, produced thereby, after standing over nignt, was filtered by suction and collected on a porous plate. Yield: about 50 g. in average. The dark gray precipitate was now dissolved in dilute acetone and decomposed by adding gradually with the saturated solution of baryta until the solution reacted very slightly alkaline. The precipitate of barium phosphotungstate was filtered off by suction immediately. After acidifying it with sulphuric acid quickly and filtering from barium sulphate, the filtrate was concentrated in vacuum to remove acetone. Further the solution freed from an excess of sulphuric acid with barium chloride exactly, was concentrated to a small volume and then added with alcohol. On standing in refrigerator for a day, whereby separated out crystals which were filtered. Crude product: 9.8 g. from 2 kg. of active Oryzanin.

(8) *Hydrochloride of nicotinic acid*:— Recrystallised from dilute alcohol. Yield: 2 g. Colorless plates, readily soluble in water, sparingly in alcohol and insoluble in acetone, benzene etc. m. p. 267~268°C. (uncorr.). Kossel's and diazo-reactions were all negative. The test on pigeons showed that it was entirely inactive. (Chart. 10).

No.	Subst mg.	CO_2 mg.	H_2O mg.	C%	H%	N%	N%
(1)	5.668	9.497	2.038	45.69	3.99	—	—
(2)	5.107	8.464	1.799	45.25	3.92	—	—
(3)	4.452	7.204	1.520	45.14	3.89	—	—
(4)	5.842	0·4312 c.c. N (15.5°C 760 mm.)		—		8.72	—
(5)	5.116	0.3822 c.c. N (21.0°C 751 mm.)		—		8.75	—
(6)	7.102	6.058 AgCl		—		—	21.34
(7)	6.251	5.403 AgCl		—		—	21.63
Calc. for $C_5H_4NCO_2H$–HCl				45.28	3.81	8.81	22.10

Picrate of nicotinic acid: Its picrate forms light yellow plates, melting at 219°C. (uncorr.).

No.	Subst mg.	Vol. of N. c.c.	Temp. C	Press. mm.	N%
(1)	4.142	0.589	20°	767	15.97
(2)	4.209	0.577	20°	752	15.80
Calc. for $C_6H_5NO_2$–$C_6H_3N_3O_7$					15.91

The alcoholic filtrate from nicotinic acid was concentrated further and finally dried in vacuum over soda-lime. The resulting product was a light brown colored hygroscopic mass which cured pigeons from polynueritis in the doses of 0.5 mg. daily. (Chart. 11). Yield: 5 g. from 100g. of active oryzanin. (Lot 1 …… 22.1 g., Lot 2……24.6 g., Lot 3…… 20.2 g., Lot 4… ··27.0 g.). From its activity, this product contains 10.000 units for pigeons, i.e, the yield corresponds to about 40% of the total activity existed in active oryzanin or 13% of the same in the original rice-polishings.

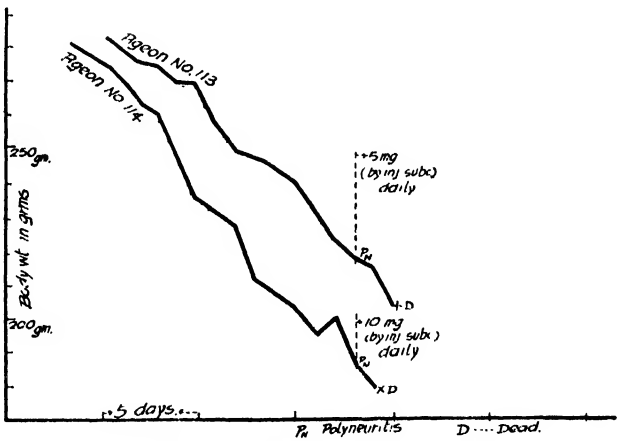


Chart. 10—Pigeons on polished rice (8) Hydrochloride of Nicotinic acid.

The product was now treated with absolute alcohol, only a small part remaining undissolved. The light brownish colored extract, after filtration, was treated with a 5% solution of platimum chloride in absolute alcohol. About 2~3 g. of platinum chloride were required usually. The orange yellow precipitate formed thus, filtered by suction after standing over night and washed with absolute alcohol.

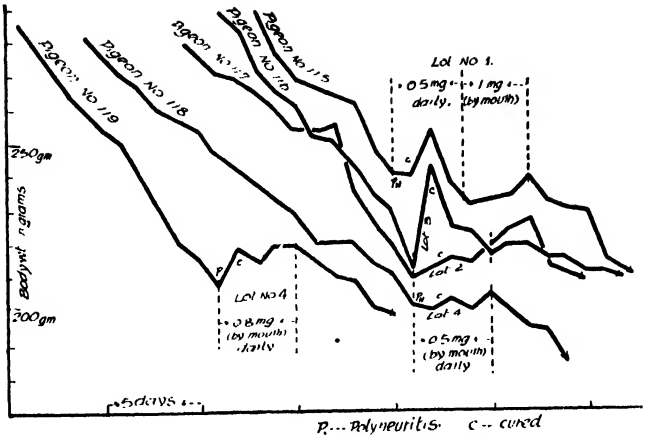


Chart. 11—Pigeons on polished rice Fraction III. ppt-phosphotuugstic acid.

i) The platinum precipitate, after being suspended in about 50 c.c. of water acidulated with few drops of hydrochloric acid, was decomposed with sulphureted hydrogen gas and filtered. The clear filtrate was evaporated to a syrupy consistency and dried in vacuum over soda-lime. The resulting hygroscopic light brown mass was active for pigeons in daily doses of 0.3~0.5 mg. (Chart. 12). Yield: 2 g. in average, (Lot 1……10.53 g., Lot 2……10.4 g., Lot 3……8.06 g., Lot. 4…11.82 g.) i.e. corresponded to about 25% of the active substance contained in active oryzanin or to about 8.8% of that in original rice-polishings.

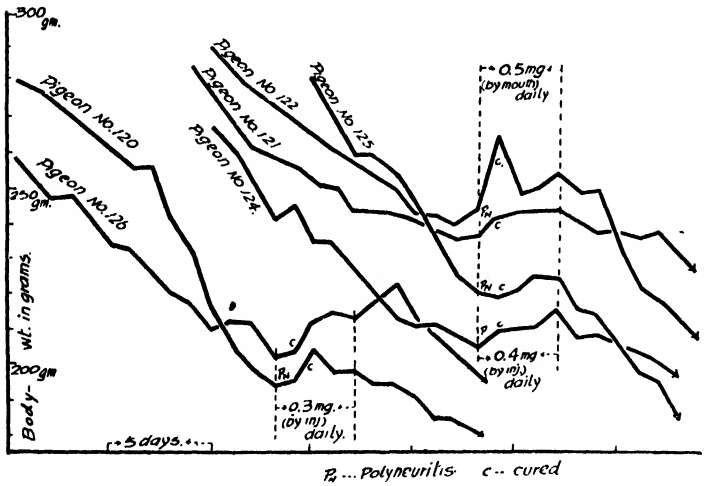


Chart. 12—Pigeons on polished rice. Fraction III. ppt-$PtCl_4$.

The product was dissolved in a small volume of absolute alcohol and filtered from a small amount of insoluble substance. For the purpose of the fractional precipitation, acetone was added cautiously to it until a white turbidity was caused. When settled over night, it subsided to the bottom forming a dark brown resinous substance which was discarded being inactive for pigeons. The solution decanted, when added with a small volume of acetone again, kept in a cool-place, separated out crystalline spherules contaminated partly with resinous substance. The supernatant fluid was decanted and spherules were collected by dissolving the resinous substance with a small

volume of absolute alcohol whereby spherules only remained undissolved. The yield of the crude product was 2.2 g. from 2 kg. of active oryzanin. (Lot 1…0.3 g., Lot 2…1.02 g., Lot 3…0.3 g., Lot 4…0.1 g.).

(9) *Unkonown base hydrochloride.* $(C_3H_6N_2\text{–}HCl)_n$: — Recrystallised from dilute alcohol. Yield: 1.7 g. It forms colorless thick plates, readily soluble in water, sparingly in absolute alcohol, melting at 267°C (uncorr.) with decomposition. Diazo–, biuret–, and purin– reactions are all negative.

No.	Subst mg.	CO_2 mg.	H_2O mg.	AgCl mg.	C %	H %	N %	Cl %
(1) Lot No. 1	4.767	5.829	1.897	—	33.35	4.42	—	—
(2) "	6.419	7.961	3.133	—	33.82	5.42	—	—
(3) "	4.715	0.985 c.c. N (16°C 769 mm.)			—	—	24.97	—
(4) "	3.276	0.676 c.c. N (16°C 771 mm.)			—	—	24.93	—
(5) "	4.975	1.020 c.c. N (16°C 761 mm.)			—	—	24.34	—
(6) "	6.288	—	—	7.766	—	—	—	31.19
(7) "	6.161	—	—	7.723	—	—	—	31.35
(8) Lot No. 2	5.969	7.693	3.366	—	35.15	6.26	—	—
(9) "	4.239	5.455	2.350	—	35.09	6.16	—	—
(10) "	5.265	6.609	2.941	—	34.23	6.21	—	—
(11) "	5.271	6.620	2.880	—	34.08	6.07	—	—
(12) "	1.988	0.354 c.c. N (17°C 767 mm.)			—	—	21.15	—
(13) "	4.102	0.747 c.c. N (18°C 767 mm.)			—	—	21.57	—
(14) "	2.935	0.531 c.c. N (18°C 767 mm.)			—	—	21.43	—
(15) "	6.361	—	—	8.376	—	—	—	32.92
(16) Lot No. 3	4.332	5.527	2.366	—	34.79	6.07	—	—
(17) "	5.223	6.653	2.863	—	34.73	6.09	—	—
(18) "	4.721	6.010	2.555	—	34.72	6.02	—	—
(19) "	4.105	0.861 c.c. N (17°C 767 mm.)			—	—	24.93	—
(20) "	4.125	0.863 c.c. N (15°C 761 mm.)			—	—	24.86	—
Calc. for $C_3H_6N_2$–HCl					33.96	6.60	26.42	33.33

From these results, it is clear that the compound is identical with the base[4] $(C_3H_6N_2)_n$ isolated from yeast extract by the present author. This compound was entirely inactive for pigeons. (Chart. 13.).

The acetone solution, decanted from the above, was treated further with acetone

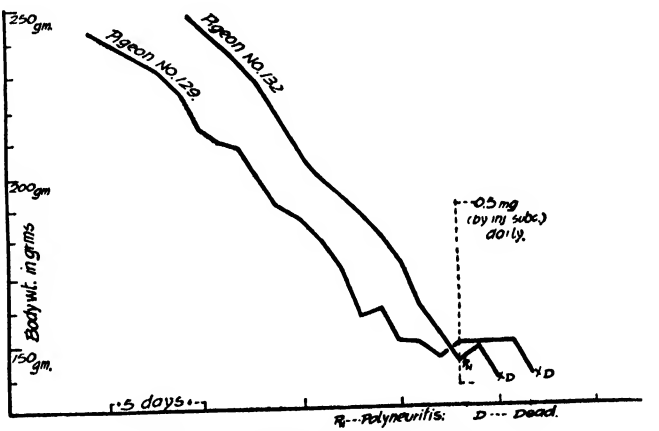


Chart. 13—Pigeons on polished-rice.
Hydrochloride of unknown base $(C_3H_6N_2\text{–}HCl)_n$.

until a white turbidity is produced and was placed in a refrigerator. These treatment was repeated day by day. After a week, crystalline spherules contaminated partly with a resinous substance separated out to the bottom and the wall of the vessel. The supernatant fluid was now decanted into another vessel and treated with more acetone, whereby a second crystallization was obtained. The crude crystals adhering to the vessel could be collected easily by treating with a small volume of absolute alcohol, the resinous substance being easily soluble in the latter. The crude crystals: 0.1 g. commonly. By treating 2 kg. of active oryzanin in this way, about 1.3 g. were obtained.

Oryzanin hydrochloride:— The crude product was now dissolved in dilute alcohol, decolorised with a small quantity of charcoal, and recrystallised by adding absolute alcohol and acetone. Yield: 0.05 g. commonly from 100 g. of active oryzanin.

It crystallises in colorless, monoclinic long plates, readily soluble in water, sparingly in absolute alcohol, but insoluble in acetone, benzene and ether etc. It gives yellowish coloration instantly which turns to red gradually when added with Pauly's diazo-reagent and gives a white precipitate by phosphotungstic acid, also by mercuric sulphate.

It contains sulphur in the form, detectable by sodium nitro-prusside or by lead acetate when it is previously boiled with alkali or fused with metallic sodium. From its melting point as well as from its activity for pigeons, it was ascertained, however, that a slight difference existed in each preparations, namely the crystals melting at 253°C (uncorr.) were only slightly active, while those melting at 220°C (uncorr.) were active. Neverthless, it was revealed by the polarization microscope that the crystals were still contaminated with some amorphous substance. Only the crystals melting at 250°C (uncorr.) were quite uniform and possesed the highest activity.

Total yield 0.85 g. from 2 kg. of active oryzanin.

Lot No.	Curde product g.	Crystals. g.	Melting Point. C.	Activity for Pigeons, tested mg.	Chart No.
1 A	0.10	0.05	237°	0.01~0.05	31
1 B	0.15	0.10	253°	0.01~0.05	32
2 A	0.05	0.03	250°	0.01	33
3 A	0.23	0.19	221°	0.01~0.025	34
3 B	0.20	0.11	244°	0.01~0.02	35
4	0.58	0.37	220°	0.01~0.04	36

Further study on this compound is described under "Properties and analysis of oryzanin hydrochloride".

The supernatant fluid decanted from the above crystals, when added with acetone more, kept in a refrigerator, separated out occationally crystalline spherules which were collected after standing for a week. Yield 0.7 g. from

2 kg. of active oryzanin.

(11) *Unknown base hydrochloride.* $(C_3H_6N_2\text{–}HCl)_n$: — Recrystallised from dilute alcohol. Yield: 0.37 g. (Lot 2···0.11 g., Lot 3···0.1 g., Lot 4···0.12 g.) Colorless plates; melting at 266°C. (uncorr.).

No.	Subst mg.	CO_2 mg.	H_2O mg.	C %	H %	N %
(1)	4.928	6.254	2.741	34.62	6.18	—
(2)	5.302	6.775	2.931	34.85	6.15	—
(3)	4.102	0.85 c.c. N (19°C 760 mm.)		—		24.24
(4)	4.018	0.82 c.c. N (19°C 760 mm.)		—		23.93
Calc. for. $C_3H_6N_2$–HCl				33.96	6.60	26.42

From its property and analytical result, it was assumed, this compound is identical with that described under (No. 9). The biological test showed it to be entirely inactive for pegions. (Chart 14).

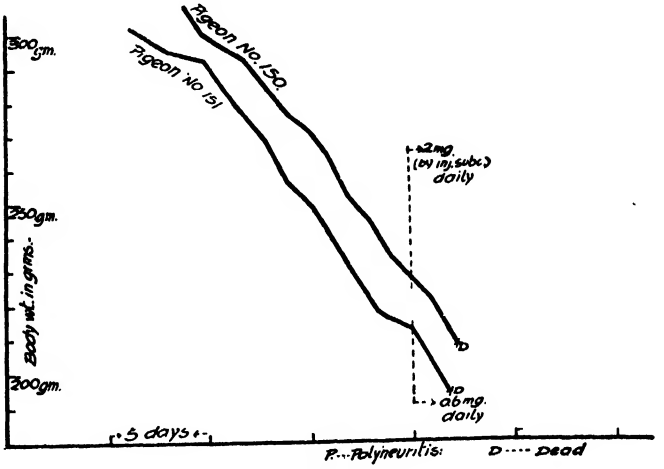


Chart. 14—Piegeons on polished rice.
(11) Hydrochloride of Unknown base. $C_3H_6N_2$–HCl

B) When the alcoholic solution filtered from (9), (10) and (11) was treated with acetone again, and a light yellow hygroscopic substance obtained, which is readily soluble in alcohol and water but insoluble in acetone. It gives intensive diazoreaction. Yield: 2 g. from 500 g. of active oryzanin (Lot. 1) Pigeon-test showed it to be of neglisible activity (Chart. 15).

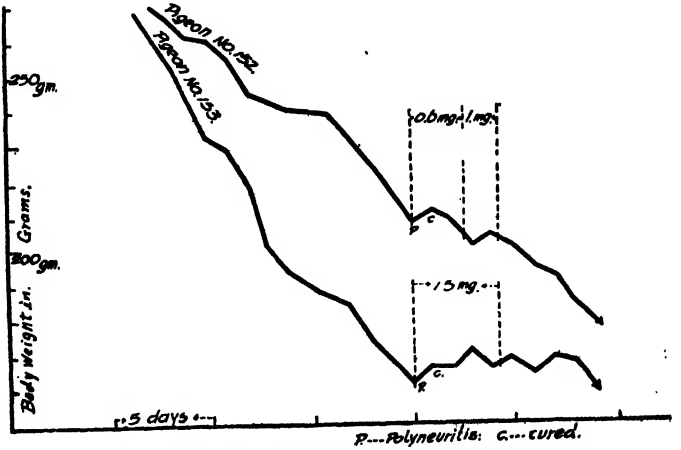


Chart. 15—Pegeons on polished rice.
Amorphous substance, insol in acetone.

C) The acetone

solution decanted from the above (A) and (B) was evaporated. The residue was dissolved again in small quantity of absolute alcohol, and filtered from a small amount of insoluble substance. By keeping it in a refrigerator for a week, hydrochloride of nicotinic acid crystallised out. Yield: 0.45 g. from 1 kg. of active oryzanin. (Lot 1. & 2.).

(12) *Hydrochloride of nicotinic acid*: — Recrystallised repeatedly from dilute alcohol. Yield: 0.17 g. Colorless plates, m. p. 266°C (uncorr.). Biological test resulted to be entirely inactive for pigeons. (Chart. 16.).

No.	Subst. mg.	CO_2 mg.	H_2O mg.	C %	H %	N %
(1)	4.874	8.114	1.752	45.41	3.99	—
(2)	4.278	7.121	1.472	45.39	3.83	—
(3)	3.942	0.292 c.c. N (18°C 760 mm.)			—	8.69
(4)	2.705	0.197 c.c. N (18°C 760 mm.)			—	8.42
Calc. for $C_6H_5NO_2$–HCl				45.28	3.81	8.81

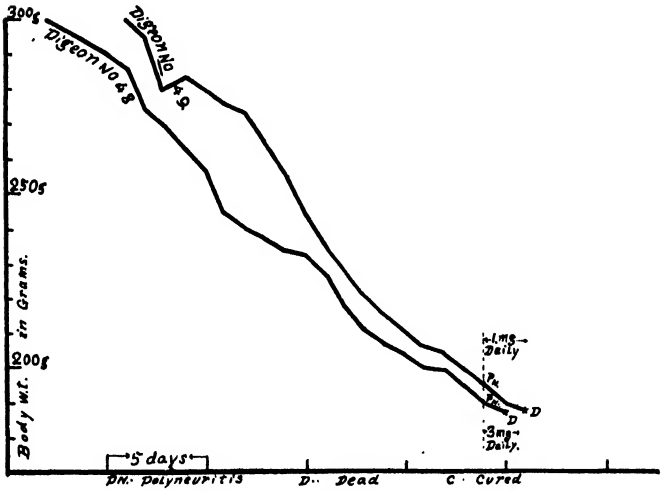


Chart. 16—Pegions on Polished rice
(12) Hydrochloride of Nicotinic acid.

The alcoholic filtrate of the above (12), which separated no more crystals by treating with acetone, was evaporated in vacuum to dryness, and about 0.8 g. of a light brown amorphous substance were obtained from 100 g. of active oryzanin. (Lot 1···4.12 g., Lot 2. & 3···7.51g.). It is readily soluble in water and alcohol, It gives an intensive diazoreaction, but it was almost inactive for pigeons. (Chart. 17.).

ii) The alcoholic solution filtered from the platinum precipitate (i) was evaporated and the residue was treated with sulphuretted hydrogen gas after suspended in water, whereby much quantity of the insoluble resinous substance separated out together with platinum sulphide which were filtered off. The filtrate was concentrated in vacuum to dryness. About 5.3 g. of a brown amorphous substance were thus obtained from Lot. 1. The product being inactive for pigeons (Chart. 18) it was proved that all active substance had been precipitated by the above platinum treatment.

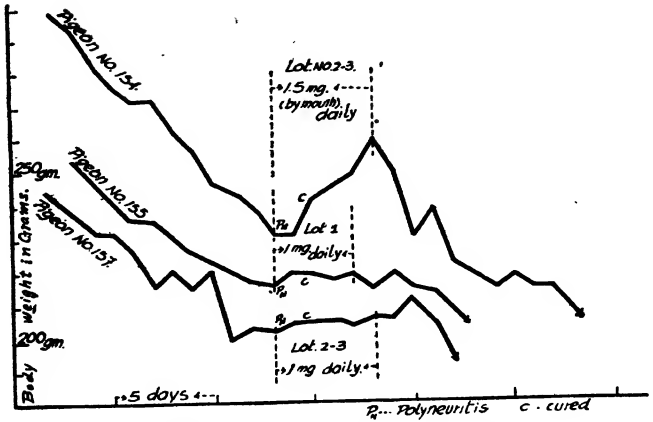


Chart. 17—Pigeons on polished rice
Amorphous substance (sol. in acetone).

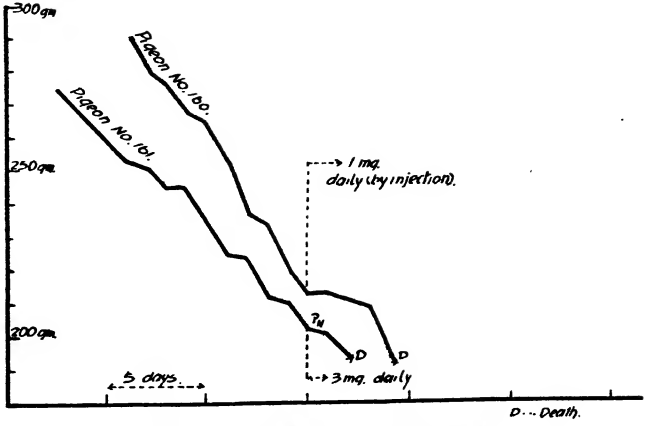


Chart. 19—Pigeons on polished rice
(13) Hydrochloride of nicotinic acid.

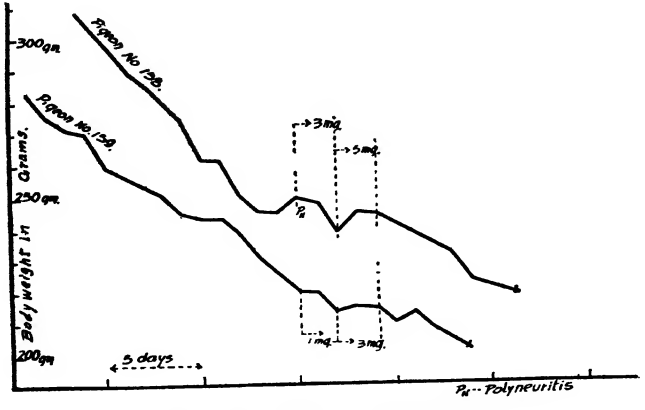


Chart. 18—Pigeons on polished rice
Amorphous substance. Filt-$PtCl_4$.

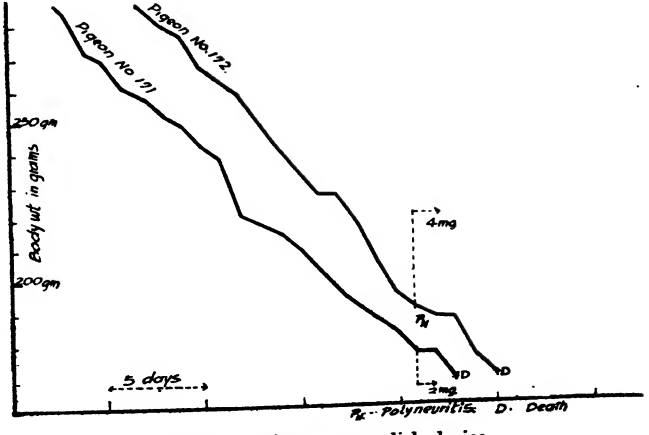


Chart. 20—pigeons on polished rice.
Amorphous substance. Filt-$PtCl_4$. Sol. in aceton.

A) The product, dissolved in a small volume of absolute alcohol, treated with acetone in the same manner as the above platinum precipitate. 1.31 g. of the crude hydrochloride of nicotinic acid were obtained. (Lot 1…0.56 g., Lot 2…0.75 g.).

(13) *Hydrochloride of nicotinic acid*: — Recystallised from dilute alcohol. Yield: 0.75 g. Colorless plates, melting at 267°C. (uncorr.). Biological test showed it to be inactive for pigeons. (Chart. 19).

No.	Subst mg.	Vol. of N. c.c.	Temp. C	Press. mm.	N %
(1)	4.010	0.311	16°	761	9.18
(2)	3.053	0.256	18°	761	9.86
(3)	6.246	0.531	20°	761	9.91
Calc. for $C_6H_5NO_2$–HCl					8.81

B) The actone solution from the above, was evaporated in vacuum to dryness and a brown amorphous substance were obtained. Yield: 3.25g. from 500 g. of active oryzanin. (Lot. 1). Although it gave strong Pauly's diazoreaction, the product was inactive for pigeons. (Chart. 20).

Fraction IV.

The silver precipitate obtained at the P_H 6.8~9.0, was decomposed by hydrochloric acid and precipitated by phosphotungstic acid inthe same manner with the preceding fraction. The phosphotungstic precipitate amounted to about 55 g. from 100 g. of active oryzanin.

The filtrate which was obtained by treating the phosphotungstic precipitate with baryta, was concentrated to a small quantity and dried in vacuum over soda-lime. Thus, 42.7g. of a light brown mass were obtained by treating 500 g. of active oryzanin (Lot. 1.). i.e. approximatly 8.5 % of active oryzanin. This product, being active in doses of 5 mg. for pigeons (Chart. 21) contains still about 6.8% of the activity existed originaly in active oryzanin or about 2.3 % of that contained in original rice polishings. The product was then treated with absolute al-

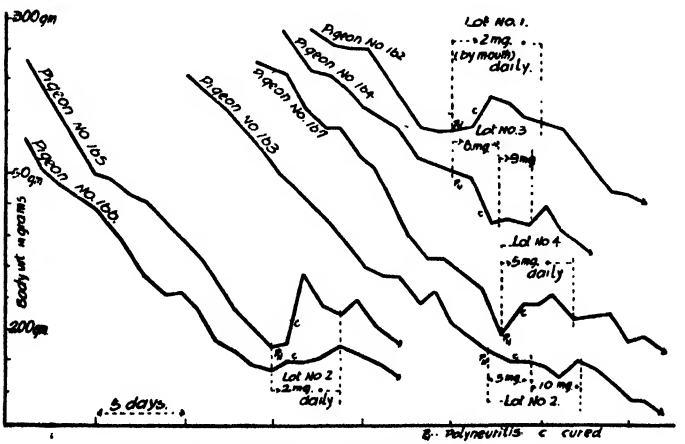


Chart. 21—Pigeons on polished rice. Fraction VI.

cohol, which dissolved the greater part and a brown substance undissolved was filtered off. The alcoholic solution was now added with a 5% solution of platinum chloride in absolute alcohol and the precipitate thus formed, was filtered by suction after standing over night in a refrigerator.

(i) The platinum precipitate which was suspended in water, decomposed by sulphuretted hydrogen gas and filtered from platinum sulphide after standing over night. The filtrate was evaporated to a small quantity and dried in vacuum over soda-lime. The resulting light brown mass weighed 12 g. from 500 g. of active oryzanin (Lot 1), and its curative daily dose was 1~2 mg. for a pigeon. (Chart 22).

The product dissolved in absolute alcohol, filtered from undissolved substance and acetone was added causiously to it in the same manner with fraction III. The solution decanted from a dark brown substance, separated out crystalline spherules when kept it in a refrigerator. The supernatant fluid was decanted and these spherules were collected by treating with small quantity of absolute alcohol. Weighed: 0.35 g. (Lot. 1).

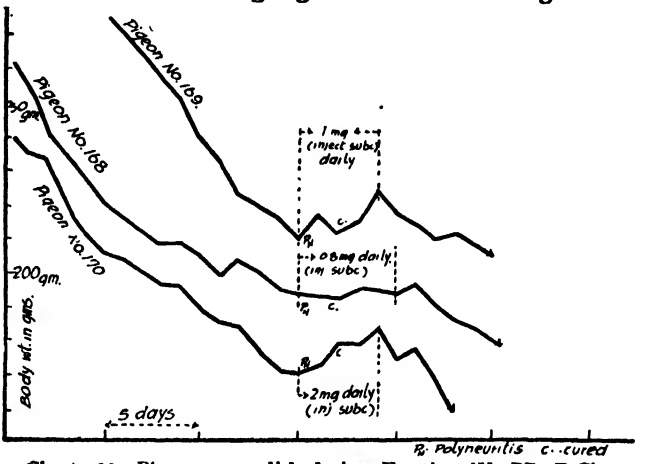


Chart. 22—Pigeons on polished rice. Fraction IV. $PPt-PtCl_4$.

(14) *Unknown base Hydrochloride.* $(C_3H_6N_2\text{–}HCl)_n$: — Recrystallised repeatedly from dilute alcohol, it forms colorless long plates, melting at 262°C. (uncorr.) with decomposition and diazo-reaction was negative. Yield: 0.2g. (from 500 g. of active oryzanin). It was inactive for pigeons. (Chart. 23).

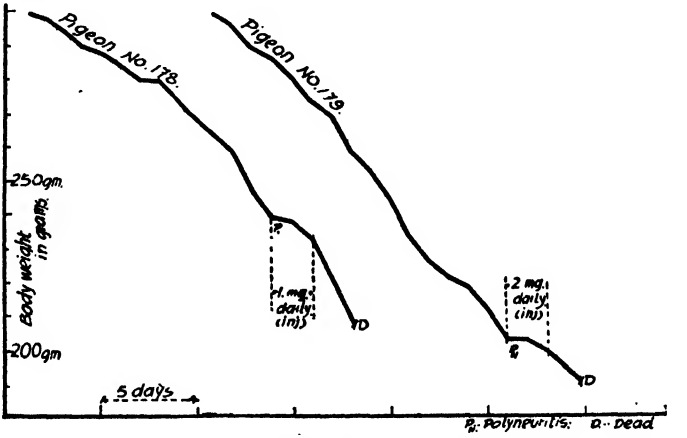


Chart. 23—Pigeons on polished rice.
(14) Hydrochloride of unknown-base $(C_3H_6N_2\text{–}HCl)$

No.	Subst. mg.	Vol. of N. c.c.	Temp. C	Press. mm.	N %
(1)	4.050	0.845	18°	763	24.57
(2)	4.711	1.000	17°	763	25.08
(3)	4.317	0.894	11°	756	24.81
(4)	5.838	1.235	12°	756	25.21
Calc. for $C_8H_6N_2$-HCl					26.42

From its properties and analytical results, it was assumed to be identical with the hydrochloride (9).

(B) The alcoholic solution filtered from preceding crystals, was treated again with acetone. The resulting hygroscopic amorphous substance, readily soluble in water and alcohol, gave intensive diazo-reaction, nevertheless it was inactive for pigeons. (Chart. 24.). Yield: 5.6 g. from 500 g. of active oryzanin (Lot 1.)

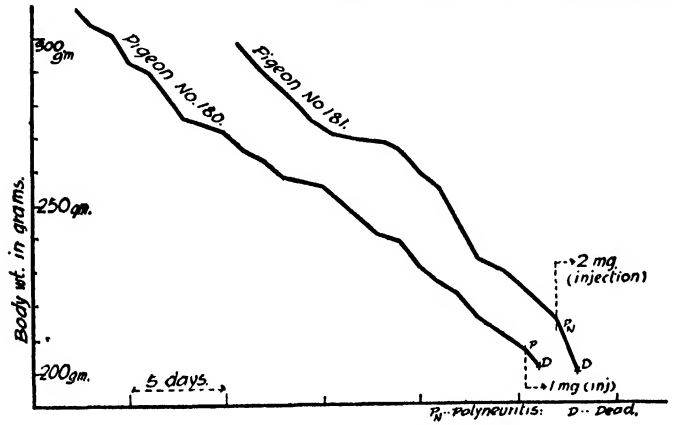


Chart. 24—Pigeons on polished rice.
Amorphous substance. Fraction VI. Sol. in acetone.

(C) The acetone solution decanted from the above fractions (A) and (B), giving no more crystalline substance by repeating the same treatment, was therefore concentrated in vacuum and dried over soda-lime. 5.6 g. of a light brown amorphous substance were obtaied from 50 g. of active oryzanin, (Lot 1). It is readily soluble in water, alcohol, and gives strong diazo-reaction. Pigeon curative test showed it to be slightly active even in daily doses of 5 mg. (Chart. 25).

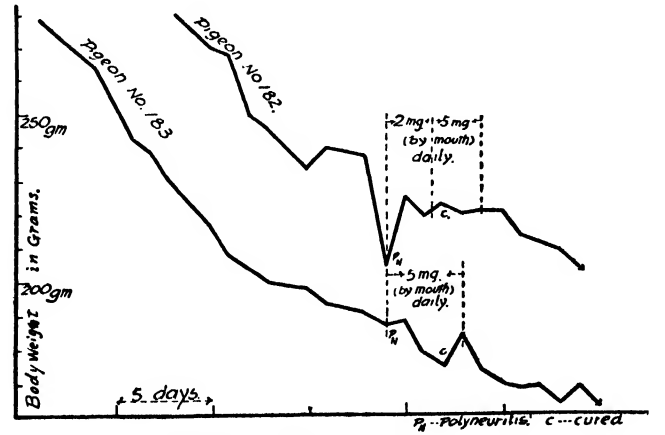


Chart. 25—Pigeon on polished rice.
Amorphous substance. Fraction VI. sol. in acetone.

(ii) The alcoholic solution filtered from platinum precipitate, was evaporated and the residue suspended in water, was decomposed by sulphuretted hydrogen gas. The filtrate was evaporated in vacuum and dried over soda-lime. The resulting mass, was extracted with absolute alcohol and filtered. By adding with acetone gradually, it separated out an amorphous substance at first and then crystalline spherules when kept in a refrigerator. The supernatant fluid decanted off and the spherules were collected by treating with absolute alcohol in the same manner with previous fraction. Yield: 1.5 g. (Lot 1.).

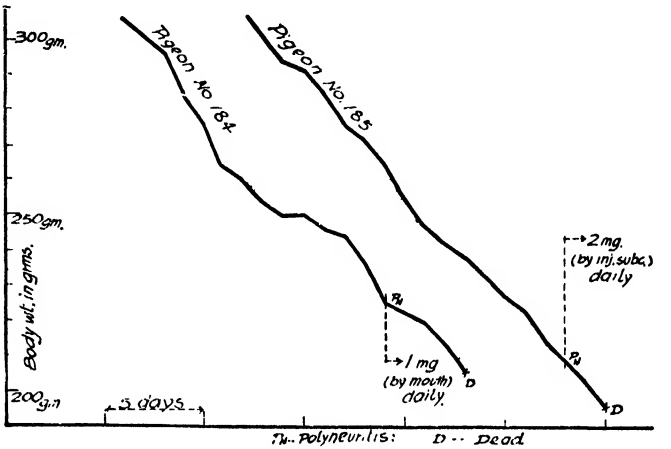


Chart. 26—Pigeons on polished rice.
(15) Hydrochloride of Nicotinic acid.

(15) *Hydrochloride of nicotinic acid*: — Those crystals, still mixed with inorganic substance, recrystallised repeatedly from hot alcohol. Yield: 0.3 g. (from 500 g. of active oryzanin. Lot. 1). Colorless plates, melting at 266 °C (uncorr.). It was inactive for pigeons. (Chart. 26).

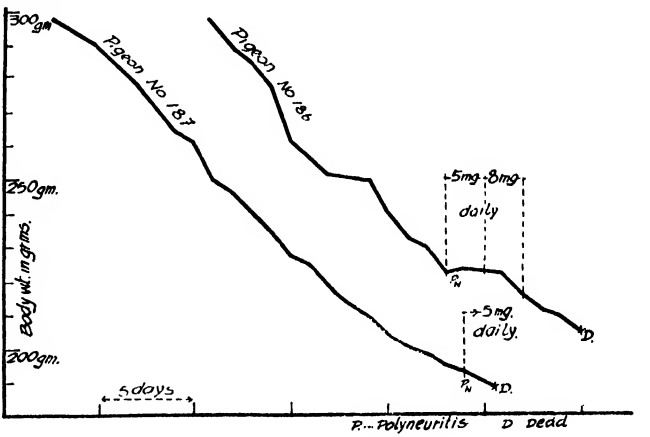


Chart. 27—Pigeons on polished rice.
Amorphous substance. Fraction IV. Filt-$PtCl_4$.

(B) The acetone solution decanted from the above which contains still solids, was evaporated and dried in vacuum over soda-lime. 2.5 g. of a light brown amorphous substance were obtained from 500 g. of active oryzanin. It gives strong diazo-reaction but pigeon curative test showed it to be inactive. (Chart. 27.).

No.	Subst. mg.	CO_2 mg.	H_2O mg.	C %	H%	N%
(1)	5.175	7.363	1.830	39.81	3.93	—
(2)	5.216	7.819	1.798	39.14	3.84	—
(3)	5.780	0.4067 c.c. N (16°C 761 mm.)			—	8.32
(4)	4.927	0.3528 c.c. N (15°C 762 mm.)			—	8.47
Calc. for. $C_6H_5NO_2$–HCl				45.28	3.81	8.81

Fraction V.

The phosphotungstic precipitate (923 g. from Lot 1.……1050 g. from Lot 2.) was dissolved in diluted acetone and decomposed by adding baryta water in slight excess. The basic solution filtered from barium phosphotungstate, was freed from an excess of baryta with sulphuric acid. After acidifying with hydrochloric acid, it was concentrated in vacuum to a small volume. it contained 232 g. of solids (116 g. from Lot 1…… 115.7 g. from Lot 2) which showed, however, to be almost inactive for pigeons. (Chart. 28.).

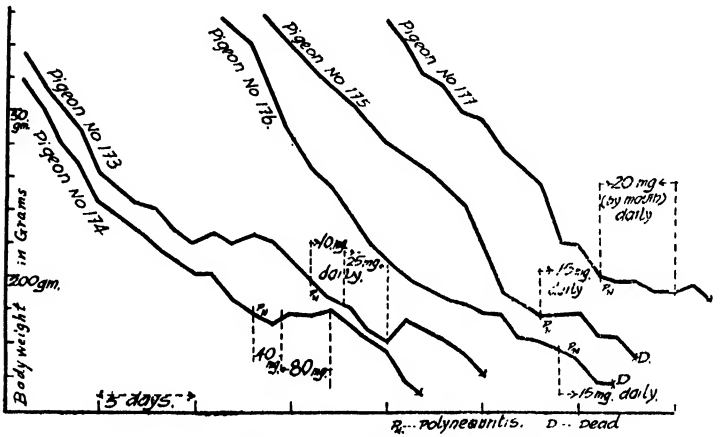


Chart. 28—Pigeons on polished rice. Fraction V.

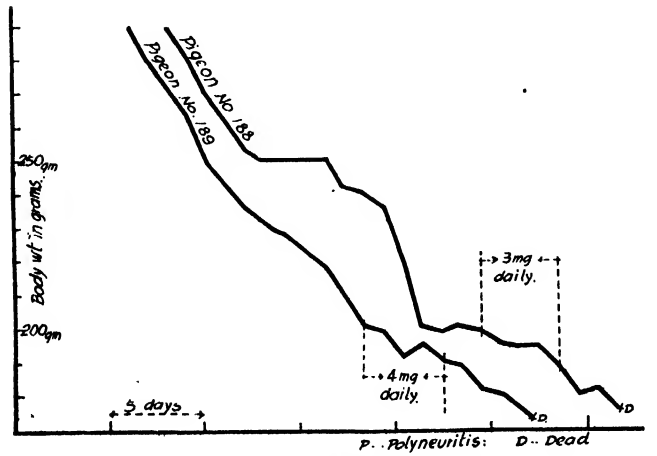


Chart. 29—Pigeons on polised rice.
(16) Hydrochloride of Nicotinic acid.

The light brownish colored solution was concentrated further to a syrupy consistence and dissolved in alcohol. On keeping the alcoholic solution in a refrigerator, a quantity of crystals separated out. The alcoholic filtrate, also

when concentrated and kept in refrigerator, gave a second crop which was collected. It amounted 44 g. (Lot 1 & 2.).

(16) *Hydrochloride of nicotinic acid*: — Recrystallised repeatedly from hot dilute alcohol. Yield: 34.8 g. (from 1 kg. of active oryzanin.). Colorless plates, readily soluble in water, sparingly in alcohol and insoluble in acetone, benzene etc. It melts at 245°C (uncorr.). and does not give diazoreaction. Pigeon curative test showed quite inactive (Chart. 29.).

No.	Subst. mg.	CO_2 mg.	H_2O mg.	C %	H %	N %
(1) Lot No. 1	5.586	9.127	2.088	44.56	4.11	—
(2) "	4.746	7.844	1.696	45.16	3.97	—
(3) "	5.039	0.374 c.c. N (14°C 755 mm.)			—	8.77
(4) "	6.101	0.441 c.c. N (16°C 756 mm.)			—	8.49
(5) Lot No. 2	4.909	8.567	2.219	47.59	5.02	—
(6) "	6.636	8.206	1.946	48.27	4.68	—
(7) "	4.962	8.671	2.079	47.85	4.65	—
(8) "	5.783	0.436 c.c. N (15°C 762 mm.)			—	8.96
(9) "	7.392	0.541 c.c. N (15°C 762 mm.)			—	8.69
Calc. for $C_6H_5NO_2$–HCl				45.28	3.81	8.81

Picrate of nicotinic acid: The hydrochloride was converted into picrate by adding picric acid to its aqueous solution. It forms light yellow thick plates melting at 219°C. (uncorr.).

No.	Subst. mg.	Vol. of N. c.c.	Temp. C	Press. mm.	N%
(1) Lot No. 1	4.076	0.539	17°	764	15.64
(2) "	5.754	0.595	17°	764	15.62
(3) Lot No. 2	5.462	0.724	17°	762	15.66
(4) "	4.388	0.580	17°	762	15.72
Calc. for $C_6H_5NO_2$–$C_6H_3N_3O_7$					15.91

The alcoholic solution filtered from above crystals was concentrated again and dissolved in absolute alcohol. On keeping the solution in a refrigerator for a week, it gave crystals in plates which were collected.

(17) *Hydrochloride of Unknown base.* ($C_8H_{10}NO_3$–HCl): — Recrystallised repeatedly from hot diluted alcohol. Yield: 4.5 g. (from 1 kg. of active oryzanin.).

It forms colorless long plates, readily soluble in water, sparingly in alcohol, insoluble in acetone, benzene, ether etc., melting at 204～205°C.(uncorr.). with decomposition. Its aqueous solution gave strong Pauly's diazo-reaction, but pigeon curative test showed it to be entirely inactive. (Chart. 30).

Its analytical results agreed with the hydrochloride of the compound having the formula $C_8H_{10}NO_3$.

Further studies of this compound is reserved.

No.	Subst mg.	CO_2 mg.	H_2O mg.	C%	H%	N%	Cl%
(1) Lot No. 1	5.387	9.018	2.276	45.66	4.69	—	—
(2) "	6.108	9.899	2.464	44.20	4.58	—	—
(3) "	4.653	7.770	2.070	45.54	4.94	—	—
(4) "	4.876	8.039	2.129	44.96	4.85	—	—
(5) "	5.432	0.3822 c.c. N (17°C 759 mm.)			—	8.30	—
(6) "	4.512	0.3283 c.c. N (17°C 760 mm.)			—	8.06	—
(7) "	5.012	0.3430 c.c. N (17°C 760 mm.)			—	8.05	—
(8) "	6.047	0.4214 c.c. N (17°C 761 mm.)			—	8.22	—
(9) Lot No. 2	6.519	11.155	3.242	46.67	5.53	—	—
(10) "	5.140	8.687	2.572	46.09	5.56	—	—
(11) "	5.159	8.783	2.646	46.43	5.69	—	—
(12) "	4.490	7.687	2.238	46.68	5.54	—	—
(13) "	5.050	0.3332 c.c. N (16°C 771 mm.)			—	7.97	—
(14) "	6.619	0.4381 c.c. N (16°C 770 mm.)			—	7.92	—
(15) "	6.576	0.4234 c.c. N (14°C 762 mm.)			—	7.61	—
(16) "	5.765	0.3773 c.c. N (12°C 763 mm.)			—	7.97	—
(17) "	5.950	3.889 mg. AgCl		—	—	—	16.34
(18) "	7.051	4.656 " "		—	—	—	16.51
Calc. for $C_8H_{10}NO_3$-HCl				47.06	5.39	6.86	17.16

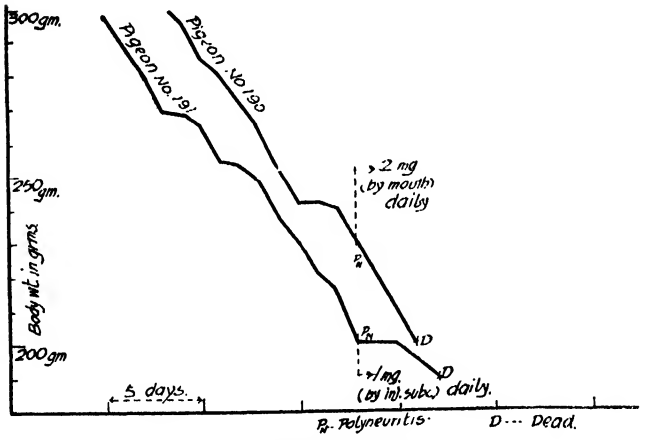


Chart 30—Pigeons on polished rice.
Hydrochloride of Unknown base $C_8H_{10}NO_3$-HCl.

The alcoholic filterate from the above crystals was evaporated in vacuum. The residue was dissolved in water and filtered from insoluble resinous substance. For the removal of hydrochloric acid in the solution, the requisite quantity of sulphuric acid to make 5% in acidity was added and precipitated again with phosphotungstic acid. The precipitate, collected by suction after standing over night, dissolved in diluted acetone, was decomposed by adding brayta water in an excess. The alkaline solution, freed from an excess of baryta, was evaporated to a small volume and added with picric acid, wherby cholin picrate separated out in yellow prisms. The filtrate, on further evaporation, separated more crystals of the same picrate. The crude picrate amounted to 13 g. (from 1 kg. of active oryzanin.).

(18) *Cholin picrate*: — Recrystallized from hot dilute alcohol repeatedly. Yield: 10 g. (Lot 1 & 2).

Light yellow prisms, melting at 246°C. (uncorr.).

No.	Subst mg.	CO_2 mg.	H_2O mg.	C %	H %	N %
(1)	4.681	6.513	3.051	37.95	5.21	—
(2)	4.754	6.741	2.113	38.67	4.94	—
(3)	5.334	0.7595 c.c. N (22°C 751 mm.)		—		16.26
(4)	5.179	0.730 c.c. N (20°C 751 mm.)		—		16.24
(5)	5.571	0.781 c.c. N (19°C 757 mm.)		—		16.32
Calc. for $C_6H_{15}NO_2$-$C_6H_3N_3O_7$				37.71	5.14	16.00

Properties and analysis of Oryzanin hydrochloride.

Crystals of Oryzanin hydrochloride, purified further by recrystallisation from alcohol and acetone repeatedly, forms colorless long monoclinic plates (Fig. 1), melting at 250°C (uncorr.) with decomposition and readily soluble in water, sparingly in alcohol, insoluble in acetone, ether, and benzene etc.

Its aqueous solution gives a white precipitate with phosphotungstic acid, or with mercuric sulphate, a dark brown precipitate with iodine potassium iodide and with Dragendorff's reagent, a yellow precipitate with picrolonic acid but not with picric acid.

It gives sulphur reaction which is detectable by giving a violet coloration with sodium nitroprusside or by giving black precipitates with lead acetate when it is boiled with alkali or fused with metallic sodium previously, while it gives no reaction with these reagents in a aqueous solution itself.

It gives Pauly's diazo-reaction showing quite different coloration to compare with that given by histidine, histamine or by thymin. When it is added with the reagent, it gives yellowish coloration instantly which turn to week redish color gradually after few minutes. It gives also a weak greenish coloration by ferriferricyanide reagent or by phosphomolybdic acid and ammonia while purin-reaction, i.e, Kossel's, Weidel's and xanthin-reactions as well as arginin reaction by α-naphtol and sodium hypochlorate are all negative.

The alcoholic solution of gold chloride and platinum chloride give its double salts which crystallises in long monoclinic plates.

The crystals, revealed to be umiform under the polarization microscope, melted at 250° and 248,5.°C. (uncorr) and were subjected to analysis.

Analysis of the hydrochloride: —

No.	Subst mg.	CO_2 mg.	H_2O mg.	C %	H %	N %	Cl %	S %
(A) M. p. 250.°C								
(1)	4.230	6.286	2.090	40.53	5.49	—	—	—
(2)	4.383	6.455	2.194	40.17	5.56	—	—	—

(3)	2.859	0.379 c.c. N (16°C 755 mm.)				15.55	—	—
(4)	2.734	0.365 c.c. N (15°C 757 mm.)				15.75	—	—
(5)	4.311	3.718 mg. AgCl		—	—	—	21.32	—
(6)	5.374	3.614 mg. $BaSO_4$		—	—	—	—	9.23
[B] M. p. 248,5°C								
(7)	5.412	8.225	2.714	41.44	5.57	—	—	—
(8)	5.235	7.949	2.471	41.41	5.25	—	—	—
(9)	4.385	0.604 c.c. N. (15°C. 757 mm.)				16.26	—	—
(10)	4.915	0.676 c.c. N. (12°C. 752 mm.)				16.29	—	—
(11)	5.499	4.413 mg. AgCl		—	—	—	19.84	—
(12)	5.261	4.236 " "		—	—	—	19.91	—
(13)	6.031	3.996 mg. $BaSO_4$		—	—	—	—	9.10
(14)	5.668	3.758 " "		—	—	—	—	9.11
Calc. for $C_{12}H_{16}N_4SO_2$-2HCl				40.91	5.11	15.91	19.89	9.09
Calc. for $C_6H_{10}N_2O$-HCl by Jansen & Donath				44.31	6.77	17.23	21.84	—

From these results, the antineuric crystals isolated by the author, is apperently the hydrochloride of a new sulphur compound having the emperical formula $C_{12}H_{16}N_4SO_2$ which does not agreed with that of Jansen and Donath unfortunately.

Activity of Oryzanin hydrochloride.

The antineuritic activity of the hydrochloride isolated was tested upon both pigeons and white rats with the following results: —

[A] *Pigeons*:

(1) Pigeons when suffering from polyneuritis by exclusive feeding on polished rice, were injected daily with 0.01 mg. of the crystals before death. The symptoms were improved in a few hours and cured completely in 1~2 days. (Chart. 31, Fig. II). In daily doses of 0.02 mg. or more, recovery is more rapid and the main symptoms disappeared in 2~3

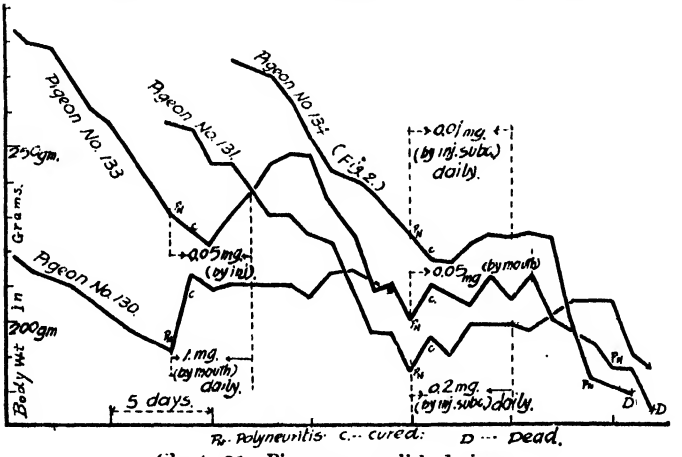


Chart. 31—Pigeons on polished rice.
Oryzanin hydrochloride. (Lot No. 1, A).

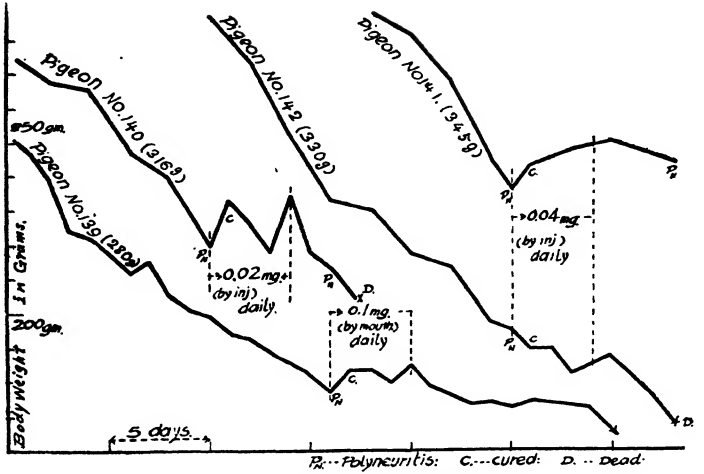


Chart. 32—Pigeons on polished rice.
Oryzanin hydrochloride. (Lot No. 1. B)

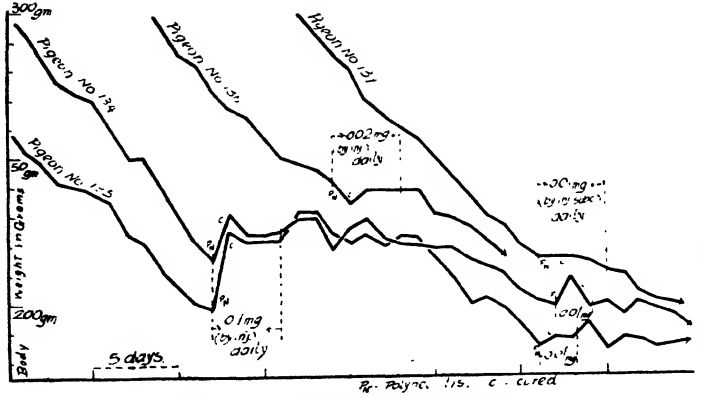


Chart. 33—Pigeons on polished rice.
Oryzanin hydrochloride. (Lot No. 2)

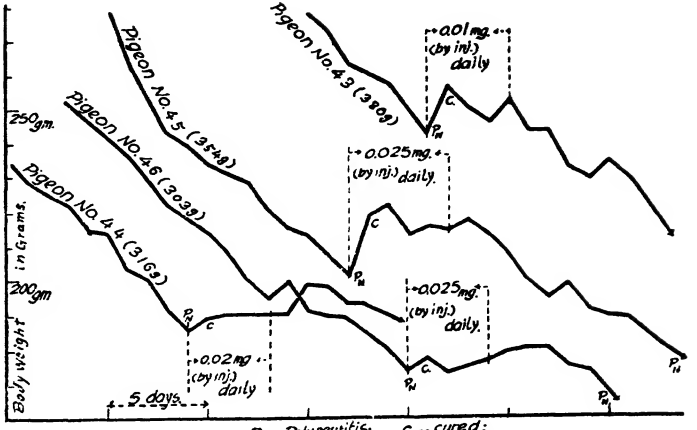


Chart. 34—Pigeons on polished rice. Oryzanin hydrochloride. (Lot No. 3)

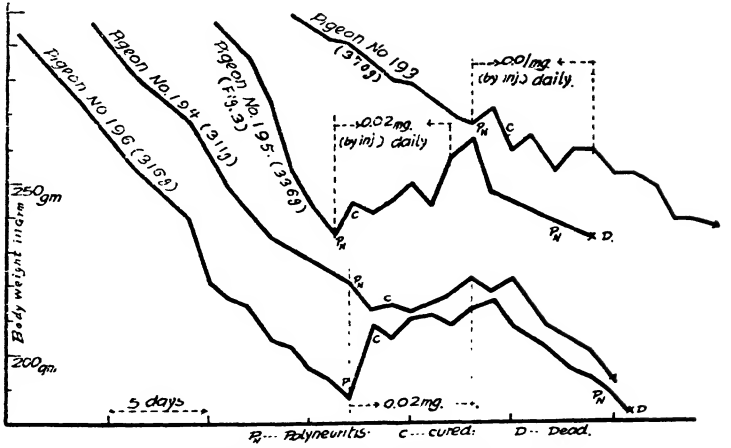


Chart. 35—Pigeons on polished rice.
Oryzanin hydrochloride. (Lot No. 3. B)

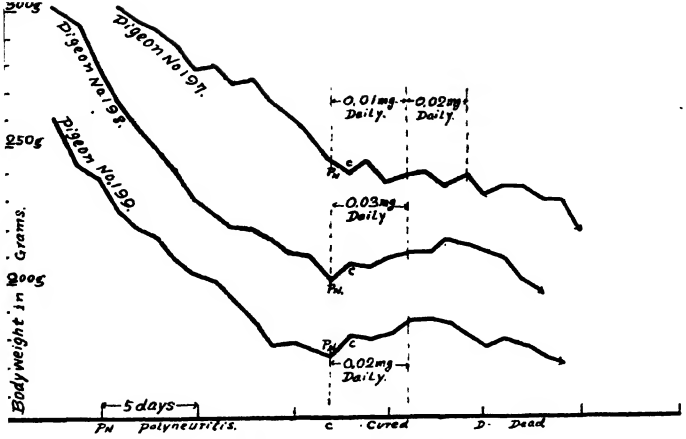


Chart. 36—Pigeons on Polished rice. Oryzanin hydrochloride (Lot No. 4)

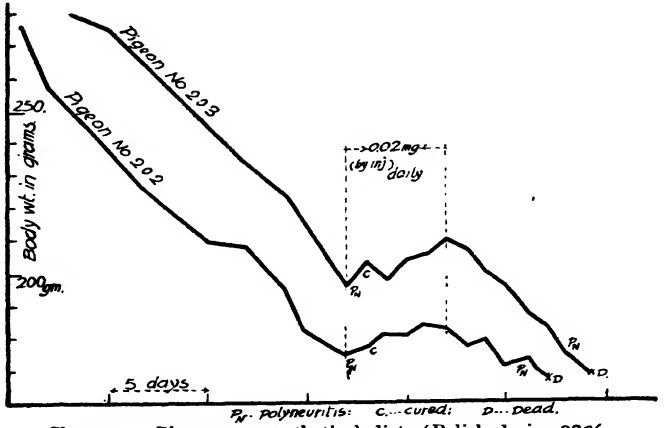


Chart. 37—Pigeons on synthetical diet. (Polished rice 92% meatprotein 5% McCollum salt 3%) Oryzanin hydrochloride.

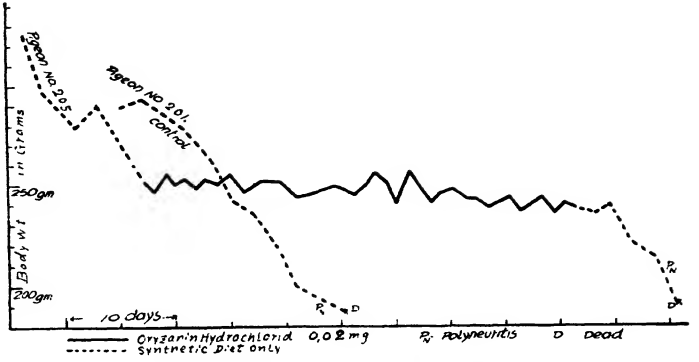


Chart. 38—Pigeons on synthetical diet. (Polished rice 92% meatprotein 5% McCollum salts 3%) Oryzanin hydrochloride.

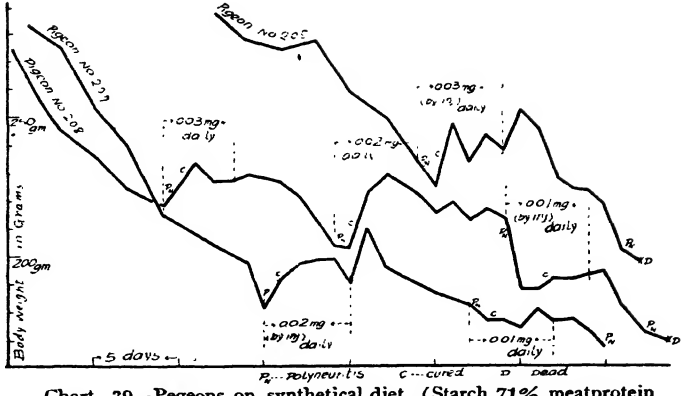


Chart. 39—Pegeons on synthetical diet. (Starch 71% meatprotein 15%. Butter 10% McCollum salts 4%) Oryzanin hydrochloride. (Lot No. 3)

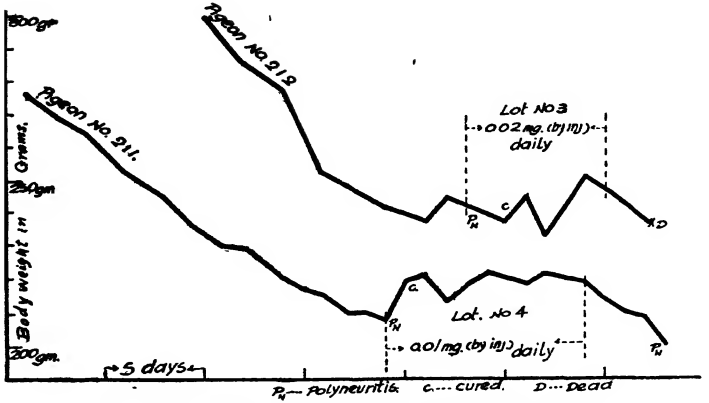


Chart. 40—Pigeons on synthetical diet. (Starch 71% meat-protein 15%, Butter 10% McCollum salts 4%). Oryzanin hydrochloride. (Lot No. 3 and 4).

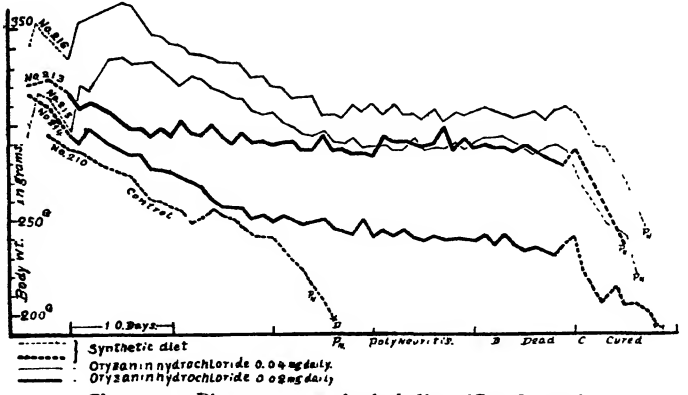


Chart. 41—Pigeons on synthetical diet. (Starch 71%, meat-protein 15%, Butter 10% McCollum salts 4%.) Oryzanin-hydrochloride.

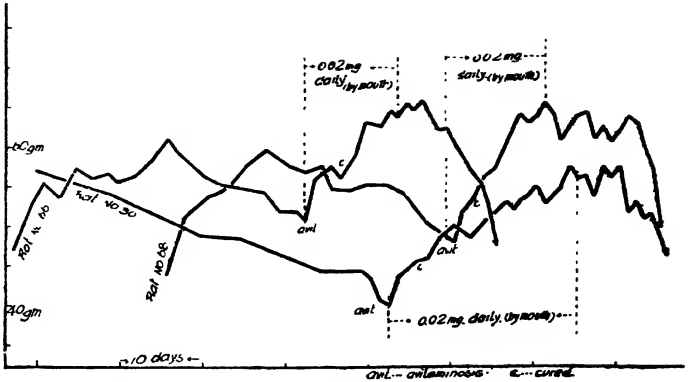


Chart. 42—Rats on synthetical diet. (Starch. 70% meat-protein 15%, Butter 10% McCollum salts 5%), Oryzanin hydrolhloride.

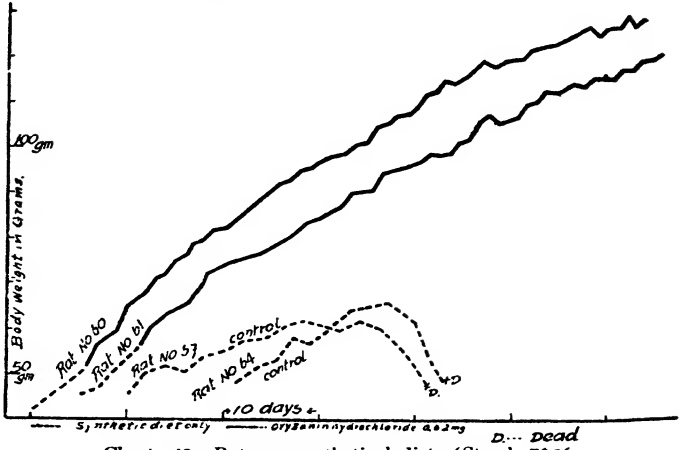


Chart. 43—Rats on synthetical diet. (Starch 70% Meat-protein 15%, Butter 10% McCollum salts 5%) Oryzanin hydrochloride.

hours. (Chart. 31～36, Fig. III).

(2) Pigeons when fed previously on the synthetical diet, consisting of 92% polished rice, 5% meat protein or extracted meat[6] and 3% McCollum salts mixture[8], developed the symptoms of polyneuritis in 3～4 weeks. By injection of 0.02 mg. daily, the main symptoms were improved within 2～5 hours and cured entirely in 1～2 days (Chart. 37.)

(3) Pigeons fed on the same diet, supplemented with 0.02 mg. daily, remained in perfect health for 50 days against polyneuritis while the symptoms of polyneuritis developed in 10 days when the supplement was withdrawn. (Chart. 38).

(4) Pigeons, when fed previously on the synthetical diet consisting of 71% purified starch[5], 15% extracted meat 10% purified butter[7], and 4% McCollum salts mixture (No. 185), exhibited the typical symptoms of polyneuritis within 4 weeks usually. By injection of 0.01 mg. daily, the main symptoms were improved in 2～3 hours and cured in 1～2 days. In doses of 0.02 mg. or more quantity, it recovered more rapidly and increased body weight markedly to compare it with the pigeons on polished rice. (Chart. 39 & 40, Fig. IV).

(5) Pigeons, fed on the synthetical diet mentioned above, administered with 0.02 mg. of the hydrochloride per os daily, remained in perfect health for 50 days though the bodyweight declined gradually. But the signs of polyneuritis were exhibited in 10 days when the supplement was removed. The supplement of 0.04 mg. daily gave also the same result. (Chart. 41).

[*B*] *White rats*:

(6) Young rats about 40～50 g. in weight, fed on the synthetical diet consisting of 80% purified starch, 15% extracted meat, 10% purifiecd butter, 5% McCollum's salts mixture and with the addition of 3 drops of codliver oil daily, exhibited the symptoms of neuritis in 4 weeks commonly. By supplementing with 0.02 mg. daily, they were cured quickly and their growth-curve paralleled almost to the normal gaining about 1～2 g. daily. The symptoms developed again on removing the supplement. (Chart. 42, Fig. V & VI).

(7) Young rats, fed on the same diet as above, supplemented daily with 0.02 mg. of the hydrochloride from the begining of the experiment, grew normally in perfect health for 70 days, gaining the body weight in the rate 1～1.2 g. daily. (Chart. 38).

Summary.

(1) An antineuritic preparation so-colled "Active Oryzanin" was prepared by treating the aqueous extract of rice polishings with Japanese acid-clay and phosphotungstic acid as described above. Its antineuritic activity is found to be in the daily dose of 4 mg. for pigeons as well as for white rats and the

Table II. Isolation of Oryzanin (Antineuritic Vitamin.)

pigeon curative day-dose to be of the order of 0.74 mg. while it lacks the pellagra protective factor and growth promoting factor.

(2) Starting from "Active Oryzanin" the author isolated the antineuritic vitamin in crystalline state according to the process of Jansen & Donath with minor modification, besides adenin, cholin, hypoxanthin, nicotinic acid, two unknown bases $(C_3H_6N_2)_n$ & $C_8H_{10}NO_3$ etc.

The yield of the antineuritic crystals was very scanty, about 0.85 g. of the purified hydrochloride (1.31 g. of crude crystals) was isolated from 2 kg. of "Active oryzanin" which were prepared from about 6.000 kg. of rice polishings.

These results are summarised in the following schematic table II.

(3) The antineuritic compound isolated by the author, crystallises in colorless long monoclinic plates, melting at 250°C. It gives Pauly's diazo-reaction in quite different coloration to compare with that given by imidazol compound and it contains sulphur in the molecule in a form detectable by sodium nitro-prusside or by lead acetate when it is boiled with alkali or fused with metallic sodium previously. From the analytical results, it was ascertained that the compound should be the hydrochloride of a sulphur compound having the formula $C_{12}H_{16}N_4SO_2$. These principal points are not agreed with that reported by Jansen and Donath.

(4) Biological experiment on pigeons and rats showed its antineuritic activity to be in the daily dose of 0.01～0.02 mg. From its activity, the content of antineuritic substance in rice-polishings is presumed to be about 0.0003%.

The further chemical and biological studies on this compound are expected in the next report.

The author express his sincere thanks to Prof. U. Suzuki for his kind advise and encouragement throughout the work. Thanks are due to Mr. T. Yuasa of Sankyo. Co. Ltd. for kind supply of the material. The author is also indebted to Messrs. K. Kamada and T. Yamagishi for their kind assistance, both in chemical and biological experiment. (Apr. 30 th, 1931. Agricultural chemical laboratory, Tokyo Imperial University. Komaba, Tokyo).

Leitz. III×4.

Fig. I—Oryzanin hydrochloride.

[A] Suffuring from polyneuritis.

[B] Curing. 2. hrs. after injection with 0.01 mg. Oryzanin hydrochloride.

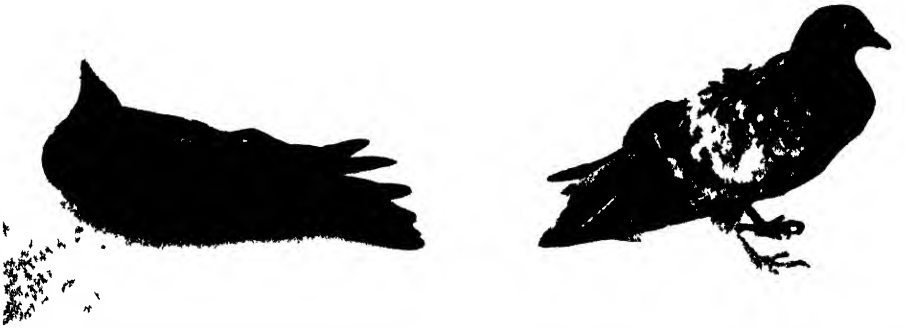
[C] Cured. 20 hrs. after injection with 0.01 mg. of Oryzanin hydrochloride.

Fig. II—Pigeons [No. 134] on polished rice. (c. f. Chart. 31.)

[A] Suffuring from polyneuritis.

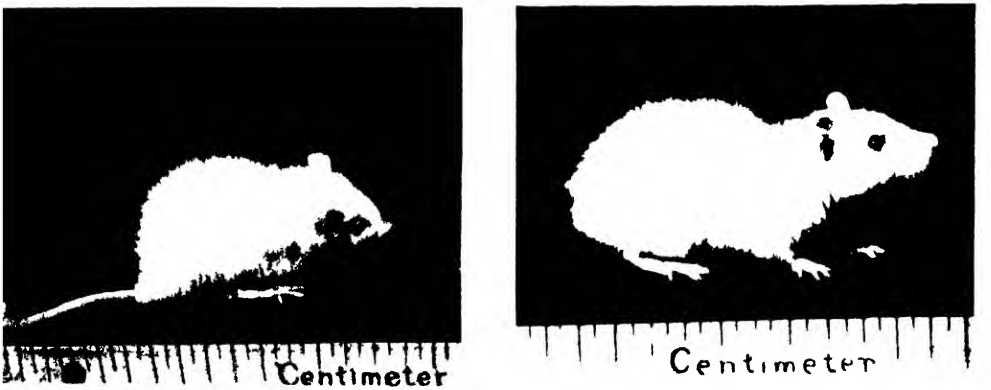
[B] 2 hrs. after injection with 0.02 mg. of Oryzanin hydrochloride.

Fig. III—Pigeons [No. 195] on polished rice. (c. f. Chart. 35.)



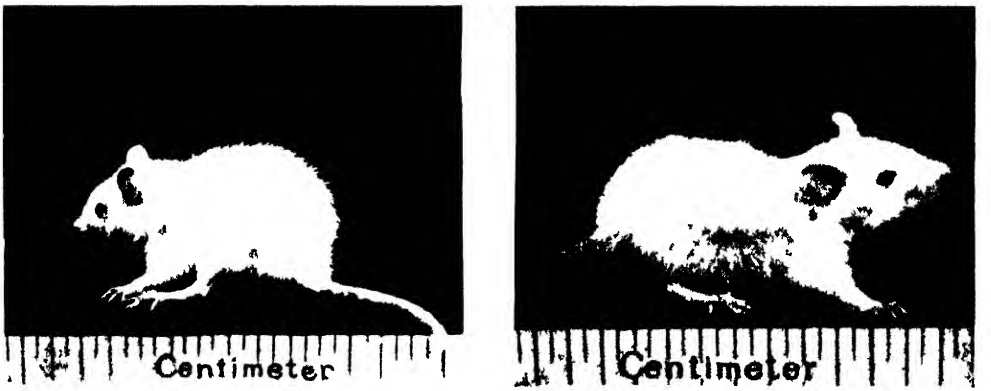
[A] Suffuring from Polyneuritis (3 weeks) [B] Cured 2 hrs after injection with 0 03 mg of Oryzanin hydrochloride

Fig IV—Pigeons (No 208) on synthetical diet (c f Chart 39)



[A] Suffering from Neuritis [B] Cured Supplement with 0 02 mg of Oryzanin hydrochloride daily

Fig V—Rat (No 66) on synthetical diet (c f Chart 42)



[A] Suffering from neuritis [B] Cured Supplement with 0 02 mg of Oryzanin hydrochloride daily

Fig VI—Rat (No 68) on synthetical diet (c f Chart 42)

The Chemical Research of the Scouring Action of Soap upon Silk.

By

Risaku TSUNOKAYE

(Received March 1, 1932)

The scouring action of soap upon silk has been studied by the author in the Laboratory of Institute of Silk Industry of the Ministry of Commerce and Industry, Yokohama, Japan. The results are as follows:

I. The scouring action of soap upon silk can be divided into two parts, first, the degumming action which is to take off the silk gum from raw silk, second, the subsequent effect of soap upon scoured silk. The degumming action of soap upon silk is concluded to the chemical action of alkali, which is developed by the hydrolysis of soap in the water solution, and also to the physical action of colloidal fatty acid soap, the so-called acid soap, which is composed from undecomposed soap and fatty acids, and it has strong adhesive power together with penetrating activity.

One of the characteristic action of soap on the scouring is to scoure silk uniformly, that is to say, the silk fibre situated inner part of the fabrics is scoured by the same time with that which is on the out side of the fabrics. This is accomplished by the strong penetrating activity of the colloidal fatty acid soap.

The alkali developed from the hydrolysis of soap, of course, has the power of dissolving sericin, the silk gum, the colloidal fatty acid soap will give negative charge to the sericin or alkali sericin when it is combined with them colloidaly and the sericin or alkali sericin will be removed by their mutual repulsive force from the fibroin.

The degumming action of soap upon silk is most effective when its concentration comes within $N/50$ and $N/70$.

When raw silk is scoured by the soap solution of $N/50$ using 50 times of its weight, the sericin will be taken off almost entirely when 16% of the soap is decomposed and consumed, but, this does not mean that 84% of soap is supra, because the undecomposed soap will play its part of scouring action by making colloidal fatty acid soap.

II. The degumming power of soap upon silk can be determined by measuring the degree of hydrolysis of its water solution.

Among several of the methods of determining the hydrolysis, "Titration Method" represents the best of all its degumming power upon silk.

Among the so-called methods of determining the power of detergency

of the soap such as I "Drop Number Test," II "Foam Number Test," and "Hydrotropie" are examined with the results that the I denotes its degumming power to some extent, but the other two methods have no relation with the power of degumming.

III. The scouring powers of several kinds of soaps are compared, the results are as follows:

A. Sodium soap of arachidic, stearic and palmitic acids have good degumming powers, while myristate, laurate, caprate and caprylate diminish their powers of degumming as well as decreasing their molecular carbon numbers.

B. The degumming powers of higher unsaturated fatty acid soaps are good as well as those of the higher saturated fatty acids, when they are fresh and not yet have undergone deterioration.

C. The soaps of hydroxylated fatty acids such as the soaps of di–, tetra–, and hexa-hydroxystearic acids, have generally weak degumming powers and the more OH groups in the molecule of fatty acid the weaker its degumming power.

D. The soaps of tallow, olive oil, sazanka oil, arachis oil, soya bean oil, cotton seed oil, chrysalis oil have quite good and the same degumming power when they are fresh, but the degumming power of soaps of cocoanut oil and castor oil are less than those of the soaps mentioned above.

E. The soaps of soya bean oil, cotton seed oil, arachis oil, are liable to go under deterioration in the atmosphere and decrease their power of scouring.

F. The characteristic property of the home made silk scouring soaps is its deterioration in the storage, the consequence of this is that they contain much soaps of unsaturated fatty acids which are easily oxidized by the atmospheric oxygen, and decrease their hydrolysis as well as degumming power.

G. The autoxidation of unsaturated fatty acid or of its soap in the atmosphere differs from the oxidation by means of chemicals such as permanganate. Na–oleate is not easily oxidized in the air. That is because the olive oil soap which is mainly composed of oleic acid is good for the purpose of silk scouring,

IV. The substances which affect the nature of the scoured silk are (I) alkali which combines chemically with fibroin, and (II) so–called acid soaps which combines physically with it. The remaining acid soap amounts about 1% of the scoured silk and when the boiled off silk is treated with acid such as acetic or others after scouring, the fatty acid occupy almost all parts of the residual substances, on the contrary, the quantity of soap will increase

when the scoured silk is treated with sodium carbonate.

A. The alkali affects the luster of the scoured silk and when its concentration is high to some extent, the silk will be coloured.

B. As the unsaturated fatty acid undergoes oxidation on the fibre of the scoured silk, the silk is liable to be coloured, but as its soap the silk is not so easily coloured, because the soap does not oxidize so easily as fatty acid on the silk fibre.

C. The scoured silk fibre, as its original nature, will produce to some degree of scroop when it is crushed in hands, but the fatty acid adsorbed on the fibre accompanied with the influence of the acid treatment makes the cause of producing the so-called scroop of the scoured silk.

The scroop produced by the saturated fatty acid is rough compared with that of the unsaturated fatty acids.

V. The silk scoured by the Na-stearate is good in whiteness of its colour, but not in luster. On the contrary, the one from Na-oleate is good in luster but the whiteness is not so good as that of the Na-stearate. The silks scoured by Na-soap of castor oil, tallow etc., which contain mainly saturated fatty acids, are good in whiteness and next come those scoured by olive oil soap and castor oil soap, but the silks scoured by the soaps of chrysalis oil, soya bean oil, cotton seed oil, arachis oil are bad in whiteness. In luster, the silk from olive oil soap is the best, those of the soaps of soya bean oil, cotton seed oil, chrysalis oil, castor oil and arachis oil come next and those of tallow soap and cocoanut oil soap are the worst. The chrysalis oil soap will produce a special bad smell on the scoured silk.

Über das Norleucin.

Von

Tokuzo YAGINUMA, Kentaro HAYAKAWA, u. Gemba ARAI.

(Eingegangen am 23, Dezem. 1931.)

Das Norleucin, welches künstlich leicht darzustellen ist, wurde in dem Naturprodukt zuerst von E. Abderhalden entdeckt[1]; nämlich gibt er an, dass er aus 5 kg der Rückenmarksubstanz der Rinder 2 g reinen Norleucin isolierte.

Zur Nachprüfung dieser Angabe haben die Verfasser nach dem Vorschrift desselben Autors den gleichen Versuch wiederholt, dessen Resultat im folgenden mitgeteilt werden soll.

(i) Das Norleucin aus Proteinhydrolysat.

5 kg der frischen Rückenmark der Rinder wurden mit konz. HCl hydrolysiert, in Äthylesterchlorhydrat übergeführt, dieses Produkt mit dem Äther überschichtet und in der Eiskälte mit KOH und K_2CO_3 behandelt. Der ätherische Auszug wurde der fraktionierten Destillation unterworfen. Nachdem der bei 100° unter 0.5 mm übergehende Teil möglichst vollkommen abdestilliert wurde, stieg der Siedepunkt verhältnismässig schnell auf 113° (0.5 mm), und das folgende Destillat ging bei eben solchen Bedingungen (113～116°; unter 0.5～0.7 mm) über, nach dessen Abdestillierung sich aber noch bei 140～150° fast nichts überdestillierte.

Die beiden Destillate wurden jedes für sich hydrolysiert, das kristallinische Produkt aus dem ersten Destillat schmeckt bitter, und liess nachweisen, dass es sich fast ausschliesslich aus *l*-Leucin besteht.

Dasjenige aus dem zweiten Destillat wurde der fraktionierten Kristallisation aus Wasser unterworfen, wodurch das 1, 2 und 3 Kristallisat nach einander erhalten wurde.

Das 1. Kristallisat (0.9 g) schmeckt süss, während das 2. und 3. immer weniger süsser war mit dem mehr stärker werdenden bitteren Nachgeschmack.

Wir untersuchten zuerst das 1. Kristallisat.

F. P. 276～278° (F. P. des *l*-Leucins nach d. Lit. 293～295°; F, P. des *d*-Norleucin nach d. Lit. 280～282°).

	C %	H %	N %
gefunden	54.60	10.04	10.76
berechnet	54.96	9.92	10.69

Durch die Bestimmung der Brechungsindizes und der Kantenwinkel[2] konnte man beweisen, dass die meisten Kristall individualisieren Norleucin (Fig. 1), während daneben einige Kristalle des Leucins vorhanden waren. Nachdem dieselbe Substanz in die Formylverbindung übergeführt wurde, beobachatete man unter dem Polarisationsmikroskop nur einen und einzigen Kristallart, nämlich denjenigen des Formyl–norleucins.

(ii) Das synthetische Norleucin.

d– und *l*–Norleucin wurde nach der Literaturangabe[3] dargestellt und gespaltet zu beiden opt. Komponenten:

$$n\text{-Capronsäure} \xrightarrow{Br} \text{Bromcapronsäure} \xrightarrow{NH_3} d\text{–, } l\text{–Norleucin}$$

$$\xrightarrow{HCOOH} d\text{–, } l\text{–Formylnorleucin} \xrightarrow{\text{Brucin}} d\text{–, } l\text{–Formylnorleucin-brucinsalz} \begin{cases} \nearrow d\text{–Salz} \rightarrow \text{Formyl–}d\text{–norleucin} \rightarrow d\text{–Norleucin} \\ \searrow l\text{–Salz} \rightarrow \text{Formyl–}l\text{–norleucin} \rightarrow l\text{–Norleucin.} \end{cases}$$

d–l–Norleucin, F. P. 297°; (Nach d. Lit. 297~300°),
Amino–N: 10.68%; Theor.: 10.69%
Formyl–*d*–norleucin, F. P. 115~116°; (Lit. 114°),
$[\alpha]_D^{25°} = -19.0 \pm 1.14°$; in Alko., (Lit. $[\alpha]_D^{20°} = -15.85°$).
Formyl–*l*–norleucin, F. P. 115~116°; (Lit. 114°),
$[\alpha]_D^{25°} = +17.3 \pm 1.2°$ in Alko., (Lit. $[\alpha]_D^{20°} = +15.53°$).
d–Norleucin, F. P. 270~275°; (Lit. 275~280°),
Amino–N: 10.62%; Theor.: 10.69%
$[\alpha]_D^{25.5°} = +18.3 \pm 1.1°$ in 20% HCl, (Lit. $[\alpha]_D^{20°} = +18.56°$; 20.44°).
l–Norleucin, F. P. 275~276°; (Lit. 275~280°),
Amino–N: 10.45%; Theor.: 10.69%
$[\alpha]_D^{25.5°} = -21.5 \pm 1.1°$ in 20% HCl, (Lit. $[\alpha]_D^{20°} = -21.17°$; 20.82°).

Formyl–*l*–leucin wurde nach der gewöhnlichen Methode durch die Behandlung des *l*–Leucins aus Weizen mit der reinen Ameisensäure erhalten, F. P. 142~144°; (Lit. 139~142°).

(iii) Die Krystallkonstanten bestimmt zur Identifierung sind wie Tafel I.

Die Krystallkonstanten des Formyl–norleucins und des Formyl–leucins wurde bestimmt zum erstenmal.

An Herren Dr. Prof. U. Suzuki und Dr. G. Takahashi mit den herzlichsten Dank für ihre Leitungen und Hinweisen.

(Aus dem chem. Laboratorium der Nihon Daigaku, Tokyo.)

Literaturausgabe

(1) E. Abderhalden u. A. Weil: H. 81 (1912), 213~217; H. 84, 49 (1913); E. Abderhalden, Fröhlich u. Fuchs: H. 86, 454 (1913).

ERRATA.

Vol. 8, Nos. 1–3 Teizo TAKAHASHI and Toshinobu ASAI.

On the Gluconic Acid Fermentation. Part III—IV.

Page	Line	Error	Correct
9	22	Beijerinck's solution.	Beijerinck's and Pasteur's solution.
〃	23	Bact. aceti Pasteur or Bact.	Bact. aceti Pasteur by the former character or Bact.
〃	24	rancens group after···	rancens group by the latter character after···
10	24	It makes no growth	It makes only poor growth···
〃	25	···solution but···	solution without giving any film but···
〃	26	By this property···	By the former property it could not belong to Bact. aceti Pasteur group and by the latter property···
11	12	in Beijerinck's or Pasteur's solution.	in Beijerinck's solution and cause just turbidity in Pasteur's solution so that it could not belong to Bact. aceti Pasteur group.

Fig. 1
d-Norleucin aus Rückenmark des Rinders
(III×5)

Fig. 2
d-Norleucin (synth.)
(III×5)

Fig. 3
l-Leucin aus Weizen
(III×3)

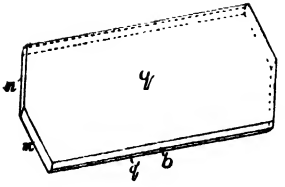


Fig. 4
d-Norleucin

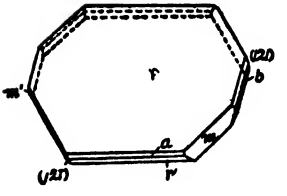


Fig. 5
l-Leucin

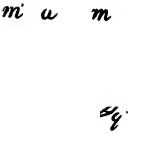


Fig. 6
Formyl-*l*-leucin

Fig. 6
Formyl-*d*-norleucin

Tafel I.

		a : b : c	α	β	γ	V	Achsenebene	I-Bis.	Dopp. brech.
Synth. *d*-Norleucin[4]	rhomb.	1.3924 : 1 : 2.8662	1.5074	1.5104	1.5400	18° 0′	{100}	[001]	Pos.
Natürl. *d*-Norleucin	〃	— — —	1.507	1.5104	1.5400	19° 1′(ber.)	{100}	[001]	Pos.
l-Leucin aus Weizen[5]	rhomb.	1.5155 : 1 : 1.0035	1.5333	1.5358	1.5512	23°20′	{100}	[001]	Neg.
l-Leucin aus Rinder	〃	— — —	1.533	1.5358	1.551	22°39′(ber.)	{100}	[001]	Neg.
Synth. Formyl-*d*-norleucin	rhomb.	0.192 : 1 : *x*	1.4833	1.5068	1.5616	34°12′(ber.)	{100}	[001]	Pos. (stark)
Formyl-*d*-norleucin aus Rinder	〃	— — —	1.4833	1.5068	1.5616	34°12′(〃)	{100}	[001]	Pos. (〃)
Formyl-*l*-leucin aus Weizen	rhomb.	0.9753 : 1 : 0.9254	1.5004	1.5070	1.5182	36°55′(ber.)	{001}	[100]	Neg.
Formyl-*l*-leucin aus Rinder	〃	— — —	1.500	1.5070	1.518	36°55′(〃)	{001}	[100]	Neg.

(2) G. Takahashi, T. Yaginuma und K. Hayakawa: Proceedings of the Imp. Acad. VII, No. 2, 57~58 (1931).
(3) loc. cit., II. 86, 454 (1913).
(4) loc. cit., P. I. A., VII, No. 2, 57~58 (1931).
(5) ibid.

Chemische Untersuchungen über das Glukomannan aus "Konjak".

VI. Mitteilung.—Über die Konstitution des Glukomannans.

Von

Kitsuji NISHIDA und Hideo HASHIMA.

(Eingegangen am 31, Jan. 1932)

Bei allen Diskussionen über Konstitutionsfragen der Cellulose und anderen polymeren Kohlenhydraten, z. B. Stärke, Lichenin, Inulin, u. a. m., begegnet man verschiedenen Überlegungen. Als Grundkörper der Cellulose stellte K. Hess die Formel $[C_6H_{10}O_5]$ auf, so dass also die Konstitution der Cellulose durch monomolekulare Glukosan-Moleküle ausgedrückt wird. Im Gegensatz zur Glukosan-Anschauung, gewann die Auffassung festere Form, danach der Grundkörper der Cellose eine Cellobiose ist (Karrer, Freudenberg u. a. m.) und der Konstitution der Cellulose die Formel $[C_{12}H_{20}O_{10}-H_2O]_x$ zukommt.

Nehmen wir also die von K. Hess aufgestellte monomolekulare Glukosantheorie für die Cellulosekonstitution als richtig an, so ergeben sich 4 artene Trihexosen oder Trihexosane und dann 3 artene Biosen oder Biosane aus Glukomannan durch Acetolyse oder andere Abbauprozesse, z. B. durch Erhitzung in Bombenröhre, durch enzymatische Wirkung, nach folgendem Schema; wenn das Glukomannan aus 2 Mol Mannose und 1 Mol Glukose besteht, dann ist:

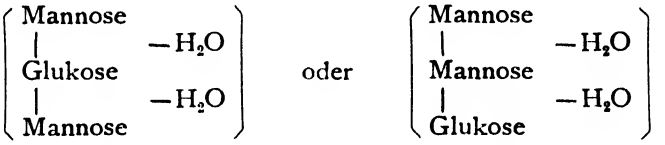
Glukomannan ⟶ { [Glukose + Mannose + Mannose]
[Mannose + Glukose + Mannose]
[Mannose + Glukose + Glukose]
[Glukose + Mannose + Glukose] } ⟶

Trihexose oder Trihexosan
(Trisaccharid)

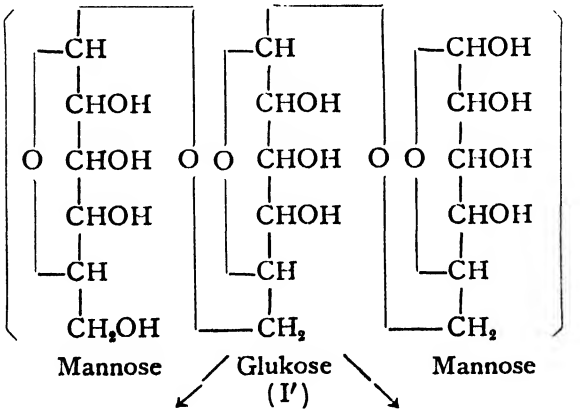
{ [Mannose + Mannose]
[Glukose + Glukose]
[Glukose + Mannose] } ⟶ { Mannose
Glukose

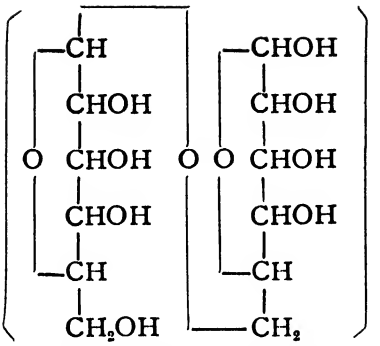
Biose oder Biosan
(Disaccharid)

Durch Acetolyse, durch enzymatische Wirkung und durch Erhitzung mit Glyzerin in Bombenröhre auf 200°C wurde nur eine artige Glukomannotrihexose od. ein Glukomannotriosan (Laevidulinose oder Laevidulin) aus Glukomannan abgespalten. Betrachtet man nun diese Tatsache, so scheint es, dass man das monomolekulare Glukosan- und Mannosan-Molekül mit gleicher Stellung des Raumes als Grundkörper des Glukomannans nicht fassen kann, aber dass 2 Mol Mannose und 1 Mol Glukose sich in ihrem eigentlichen Raume als Baustein zum Trisaccharid d. h. zur Glukomannotrihexose, vereinigen. In welcher Weise die in Betracht kommenden Mannose- und Glukosegruppen miteinander verknüpft sind, kann man aus folgendem Schema entnehmen:

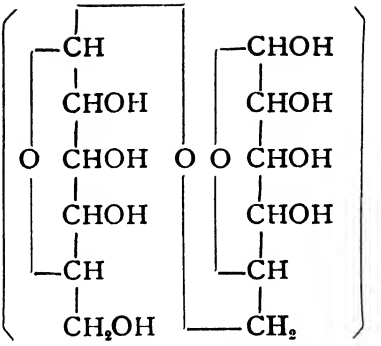


Bei der Methylierung des Glukomannans entsteht unter Eintreten von drei Methylgruppen das Trimethylglukomannan, das wurde bei der Hydrolyse mit methylalkoholischer Salzsäure in 2, 3, 4-Trimethylmannose, 2, 3, 6-Trimethylmannose und 2, 3, 4-Trimethylglukose zerspalten. Nehmen wir die von dem fünfgliedrigen Ring ausgeführte Glukose- und Mannosekonstitution als richtig an, ergibt sich für die Glukomannotrihexose die folgende Konstitution aus dem obigen Schema.

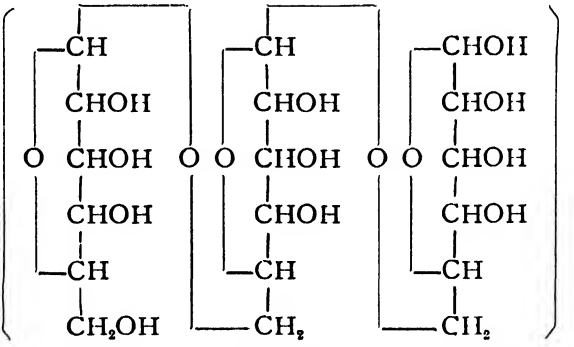




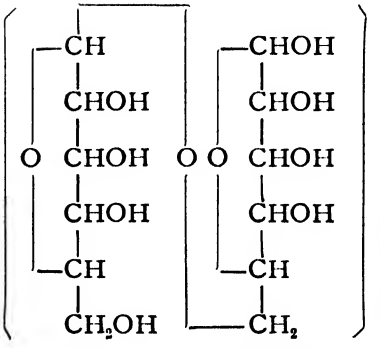
Mannose Glukose
Glukosemannosid
(III)



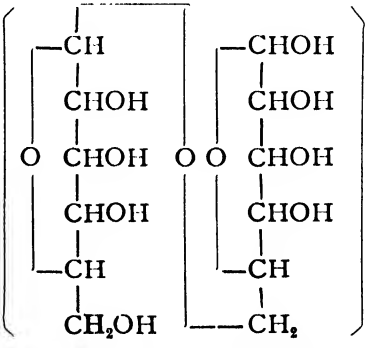
Glukose Mannose
Mannoseglukosid
(IV)



Mannose Mannose Glukose
(II′)



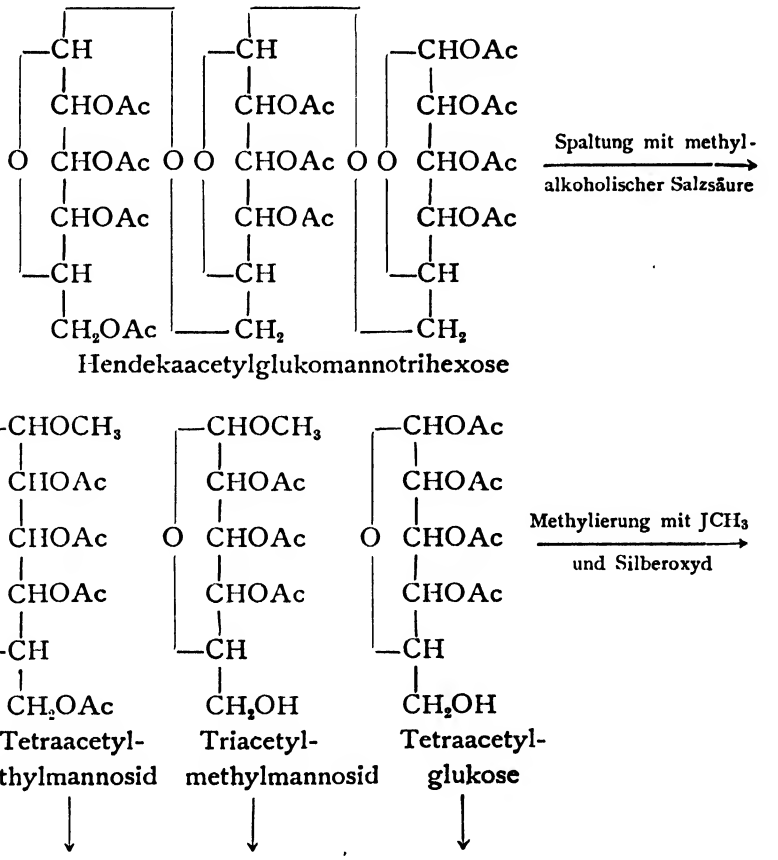
Mannose Mannose
Mannosemannosid
(V)

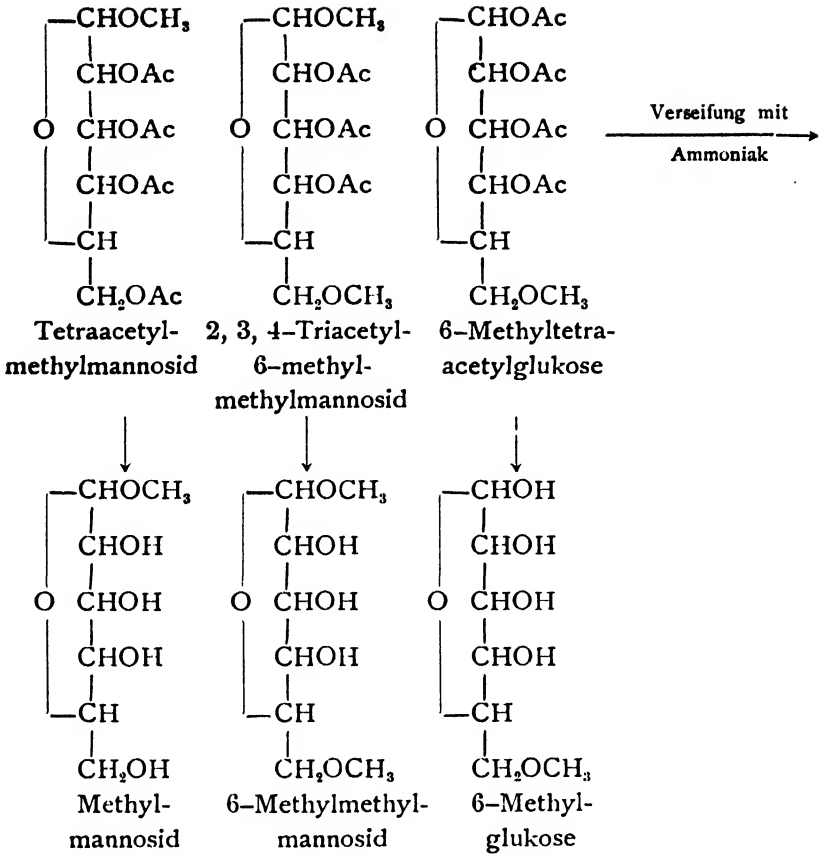


Mannose Glukose
Glukosemannosid
(VI)

Die Acetolyse des Glukomannans ist schon über den für Hendekaacetylglukomannotrihexose charakteristischen Zustand hinausgegangen und darüber zu sehr viel weiter abgebauten Stoffen (Disacchariden) von kleinerem Molekulargewicht mit höherem Acetylgehalt abgespalten worden, von deren eines (Mannosemannosid) nur die Mannose und das andere (Glukosemannosid) 1 Mol Mannose und 1 Mol Glukose hydrolysierbar ist. Darauf erfolgt die Konstitution der Glukomannotrihexose nach dem II′ Schema.

Die Stellung der Mannose und der Glukose in der Glukomannotrihexose (I′ oder II′) ist auch noch durch weitere Versuche angeführt worden, dafür Hendekaacetylglukomannotrihexose mit 1% iger Salzsäure zu Hexoseacetate gespalten wurde. Man kann die so gewonnenen Hexoseacetate mit Methyljodid und Silberoxyd methylieren, daraus mit methylalkoholischem Ammoniak verseifen. Dabei Methylmannosid, 6-Methylmetylmannosid und 6-Methylglukose nach folgendem Schema erhalten wurden.



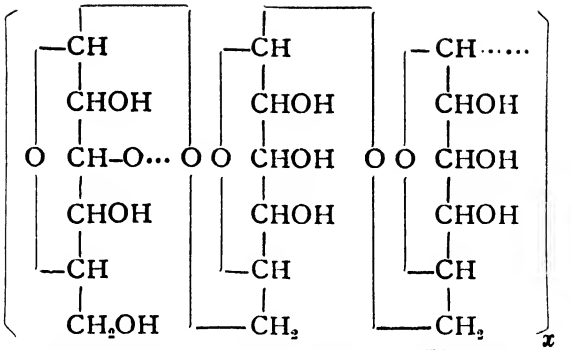


Die Konstanten aus diesen Versuchen ergaben:

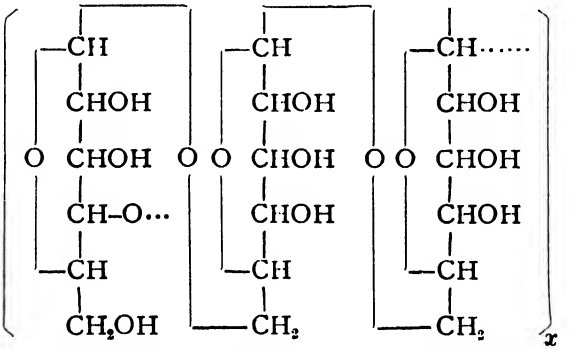
Kristall	Sirup-B	Sirup-A
Smp. 173°C	OCH_3-Gehalt gef. 30.61; 30.38	OCH_3-Gehalt gef. 21.75; 21.45
(Ausbeute war sehr gering, kristallisierte nur einmal.)	OCH_3-Gehalt berechnet für 6-Methylmethylmannosid 30.77	OCH_3-Gehalt berechnet für 6-Methylglukose 15.98
	$[\alpha]_D^{20}$ in Alkohol +70.0 $[\alpha]_D^{20}$ in Alkohol +54.0*	$[\alpha]_D^{20}$ in Alkohol +86.9°
	$[\alpha]_D^{20}$ in Wasser +61.5 $[\alpha]_D^{20}$ in Wasser +59.6*	$[\alpha]_D^{20}$ in Wasser +75.8°

* Endwert

Für dieses Ergebnis gibt es nur wenig stichhaltige Argumente, wir konnten daher nicht näher darauf eingehen. Aber aus der oben beschriebenen Acetolyse und Methylierung bestehen für die Konstitution des Glukomannans die folgenden beiden Möglichkeiten (VII und VIII).



(VII)



(VIII)

Gemäss der neuen Formulierung für Cellubiose und Maltose traten diese als (VIII) Formel ein.

Über die Oberflächenaktivität und die Adsorbierbarkeit von Aminosäuren.

III. Mitteilung.

Von

Takeo Ito.

(*Aus dem Agrikulturchemischen Laboratorium der Landwirtschaftlichen Hochschule Morioka.*)

(Eingegangen am 20, Marz 1932.)

Die Aminosäuren sind bekanntlich amphotere Elektrolyte, indem ihre

Aminogruppe als Base funktioniert, ihre Karboxylgruppe als Säure. Durch Säurezusatz wird ihre Säuredissoziation zurückgedrängt und die Aminosäurekationen werden infolge der Salzbildung vermehrt. Umgekehrt wirkt ein Laugezusatz. Der Dissoziationsrest ρ einer Aminosäure in wässeriger Lösung, d. h. das Verhältnis der Konzentration der neutralen Aminosäuremoleküle zur Gesamtkonzentration, wird bestimmt durch die Gleichung

$$\rho = \frac{[H_2N\cdot R\cdot COOH]}{[H_2N\cdot R\cdot COOH]+[H_3^+N\cdot R\cdot COOH]+[H_2N\cdot R\cdot COO^-]}$$

$$= \frac{1}{1+\frac{k_a}{[H^+]}+\frac{k_b}{k_w}[H^+]} \qquad (1)$$

worin k_a die Säure- und k_b die Basendissoziationskonstante der Aminosäure bedeuten, k_w die Dissoziationskonstante des Wassers. Gemäs dieser Gleichung wird ρ ein Maximum bei der $[H^+]=\sqrt{\frac{k_a}{k_b}k_w}$ zeigen, also im isoelektrischen Punkt, wo $[H_3^+N\cdot R\cdot COOH]=[H_2N\cdot R\cdot COO^-]$ ist. (L. Michaelis, Die Wasserstoffionenkonzentration I., 2. Aufl., 1922).

Nun es ist auf Grund der bisherigen Erfahrungen im allgemeinen erkann, dass in wässeriger Lösung die Fettsäuren oder die Aminen wesentlich höher oberflächenaktiv sind als ihre Salze. Demgemäss ware zu erwarten, dass eine Aminosäure lösung bei der konstant gehaltenen Konzentration und der wechselnden Wasserstoffionenaktivität im isoelektrischen Punkt am stärksten oberflächenaktiv sein muss, denn sie enthält gerade in diesem Punkt den maximalen Anteil der undissoziierten Aminosäuremoleküle. Die vorliegende Untersuchung lehrt aber, dass die Oberflächenspannung der wässerigen Lösungen der oberflächenaktiven Aminosäuren auf der stark sauer Seite am niedristen liegt, um sich sodann mit abnehmender H^+–Ionenaktivität zu erhöhen. Es folgt demnach, vom Standpunkt der erwähnten Anschaungsweise betrachtet, dass die Oberflächenaktivität in der folgenden Reihenfolge fällt:

$$H_3^+N\cdot R\cdot COOH > H_2N\cdot R\cdot COOH \geqq H_2N\cdot R\cdot COO^-,$$

was mit der erwähnten Erkenntnis, dass die undissoziierten Moleküle der schwachen Elektrolyte wesentlich höher oberflächenaktiv sind als ihre Ionen, nicht im Einklang stehen würde.

N. Bjerrum hat es neuerdings als wahrscheinlich erwiesen, dass die aliphatischen Aminosäuren im undissoziierten Zustande fast ausschliesslich als salzartige Zwitterionen ($H_3^+N\cdot R\cdot COO^-$) vorhanden sind (Z. physik. Chem. 104, 147, 1923). Die Dissoziationsprozesse einer Aminosäure werden nach dieser Anschaung im Sinne der folgenden Gleichungen dargestellt:

$$H_3^+N\cdot R\cdot COOH = H_3^+N\cdot R\cdot COO^- + H^+ \qquad (2)$$

und $$H_2O + H_2N{\cdot}R{\cdot}COO^- = H_3^+N{\cdot}R{\cdot}COO^- + OH^- \quad (3)$$

Die Säure- und Basendissoziationskonstanten, K_S und K_B, die den Gleichungen (2) und (3) entsprechen, stehen mit den alten Dissoziationskonstanten in folgenden Verhältnissen:

$$K_S = k_w/k_b \quad \text{und} \quad K_B = k_w/k_a.$$

Es ist allerdings "für die Berechnung des Gleichgewichtes zwischen Kationen, Anionen und elektrisch neutralen Molekülen gleichgültig, ob man von der älteren oder der neueren Anschauung ausgeht". So wird der Dissoziationsrest ρ, welcher numehr als das Verhältnis von Zwitterionenkonzentration zur Gesamtkonzentration aufgefasst werden muss, durch dieselbe Gleichung wie (1) angegeben. Vom Standpunkt dieser neueren Anschauungsweise betrachtet, muss die oben angeführte Bezeichungsweise für die Reihenfolge der Oberflächenaktivität korrigiert werden in

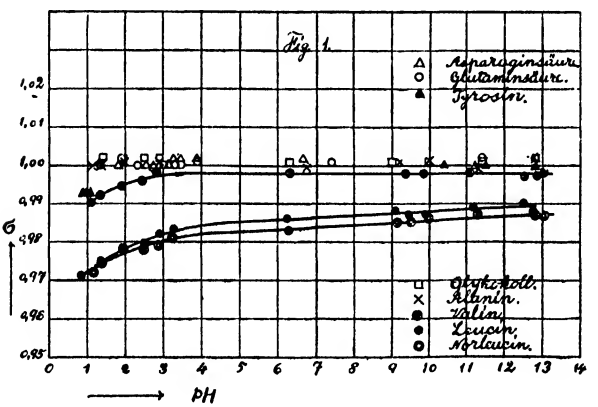
$$H_3^+N{\cdot}R{\cdot}COOH > H_3^+N{\cdot}R{\cdot}COO^- \geqq H_2N{\cdot}R{\cdot}COO^-,$$

die uns erscheint plausibler zu sein.

Für die Versuche wurden stets 0.05 mol. Aminosäurelösungen, deren H^+-Ionenaktivität durch HCl- bzw. HaOH-Zusatz variert worden war, verwendet. Die Oberflächenspannung wurde mit Hilfe von Tropfenmethode gemessen, indem man ein Stalagmometer nach Trabe (Firma C. Gerhardt) benutzte. Die H^+-Ionenaktivität wurde potentiometrisch bestimmt, indem man sich einer Chinhydronelektrode auf der sauren Seite und einer H-Elektrode auf der alkalischen Seite bediente.

Versuchstemperatur betrug $18 \pm 0.5°C$.

Die Ergebnisse sind in Fig. 1 graphisch zusammengestellt, wobei als Ordinate die relative Oberflächenspannung σ ($\sigma_{H_2O} = 1$) zugeordnet ist, als Abszisse pH.



Hieraus its zu ersehen, dass Glykokoll, Alanin, Asparagin- und Glutaminsäure die Oberflächenspannung des Wassers, unabhängig vom pH, kaum beeinflussen. Es sei in diesem Zusammenhang darauf aufmerksam gemacht, dass die sämtlichen genannten Aminosäuren in rein wässeriger Lösung als oberflächeninaktiv bestätigt worden sind (Ito: diese Zeitschr., 6, 13, 1930).

Die relative Oberflächenspannung der Lösungen von Valin, Leucin und

Norleucin ist, wie aus der Figur ersichtlich, am kleinsten auf der stark sauren Seite; mit zunehmendem pH wächst sie anfangs stark dann nur allmählich, bleibt aber stets kleiner als 1.

Aus dem Gesagten darf man erschliessen, dass die erstgenannten Aminosäuren bei einer Konzentration von 0.5 m. die Oberflächenspannung des Wassers fast gar nicht beeinflussen gleichgültig, ob sie als elektrisch neutrale Moleküle oder als Ionen vorhanden sind, während die letztgenannten dagegen sowohl im elektrisch neutralen als auch im dissoziierten Zustande oberflächenaktiv sind, und zwar in der schon erwähnten Reihenfolge.

Ferner sieht man, dass Tyrosin nur als Kation merklich oberflächenaktiv ist.

Wenn man nun die Oberflächenaktivitäten der jeden einzelnen Aminomonocarbonsäuren bei einem bestimmten pH vergleicht, so erkennt man stets die folgende Reihe:

Norleucin > Leucin > Valin > Alanin, Glykokoll.

Über die Oberflächenaktivität und die Adsorbierbarkeit von Aminosäuren.

IV. Mitteilung.

Von

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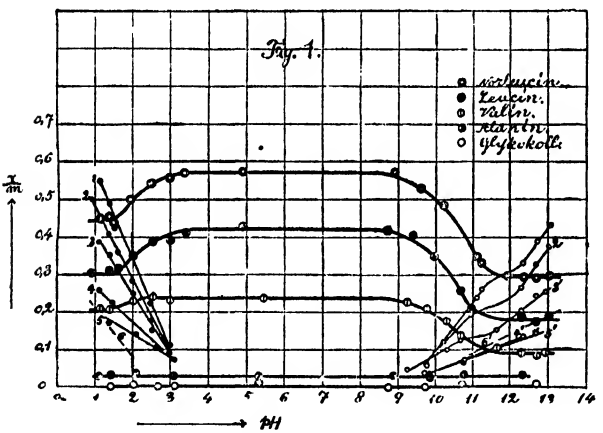
(Aus dem Agrikulturchemischen Laboratorium der Landwirtschaftlichen Hochschule Morioka.)

(Eingegangen am 20, März 1932)

Nach Fromageot und Wurmser (Compt. rend., 179, 972, 1924) gibt es keine "einfache Beziehung" zwischen der Adsorbierbarkeit der Fettsäuren an Kohle und ihren Dissoziationskonstanten, obwohl die freien Säuren bekanntlich stärker adsorbierbar sind als ihre Salze. In neuester Zeit haben Phelps und Peters (Proc. Roy. Soc. London, A. 124, 554, 1929) untersucht über die Adsorption von Fettsäuren, Aminen und einigen Aminosäuren an dem nach Miller gereinigten Norit in Zusammenhang mit der H^+-Ionenaktivität. Sie gelangen zu dem Schluss, dass die scnwachen Elektrolyte nicht als Ionen, sondern nur durch Vermittlung der undissoziierten Moleküle adsorbierbar sind. In dieser Arbeit haben wir die Beziehung zwischen der Adsorption der Aminosäuren an Kohle (E. Merck, Carbo animalis pur. sicc.; Aschengehalt =2.17%) und der H^+-Ionenaktivität untersucht. Unsere Resultate stimmen zwar mit denen der genannten Autoren insofern überein, als hier die elektrisch

neutralen Moleküle am stärksten adsorbierbar sind. Die Aminosäureionen sind jedoch, wie wir glauben, auch adsorbierbar. Dies dürfte wenigstens dann nicht überraschend sein, wenn man bedenkt, dass einige oberflächenaktive Aminosäuren ungeachtet der H^+–Ionenaktivität die Oberflächenspannung des Wassers erniedrigen, wenn auch in verschiedenem Masse (vgl. III. Mitt.).

1. Als Versuchslösungen wurden stets 0.05 mol. Aminosäurelösungen, deren H^+–Ionenaktivität durch HCl– bzw. NaOH–Zusatz variiert worden war, verwendet. Es wurden sowohl die Adsorption der Aminosäure als auch die der Cl^-– bzw. Na^+–Ionen bestimmt. 2.500 g. Kohle wurden in einen kleinen mit eingeschiffenem Glasstopfen verschliessbaren Erlenmyerkolben genau eingewogen und mit 50 cc. Versuchslösung zusammengebracht. Nach 20 Minuten Schütteln im Thermostat bei $18 \pm 0.1°C$ wurde die Kohle abzentrifugiert. Hierauf wurden die Lösungen vor und nach der Adsorption analysiert und daraus die adsorbierten Mengen der Aminosäure und des Cl^-– bzw. Na^+–Ions ermittelt. Dabei wurde die Aminosäure nach van Slyke, Chlor massanalytisch nach Volhard und Natorium gravimetrisch als Na_2SO_4 bestimmt. Gleichzeitig wurde die H^+–Ionenaktivität potentiometrisch bestimmt. In Fig. 1 sind die Resultate der Versuche mit Aminomonocarbonsäuren graphisch zusammengestellt, wobei als Ordinaten die adsorbierten Mengen in Millimol/g. Kohle, als Abszissen die pH der Gleichgewichtslösungen aufgetragen sind.



Hier stellen die dick ausgezogenen Linien die Adsorptionskurven für die Aminosäuren dar, die anderen die für Cl^-– bzw. Na^+–Ionen.

Kurve 1 bzw. 1′ zeigt den Adsorptionsverlauf des Cl^-– bzw. Na^+–Ions bei Anwesenheit von Norleucin.
Kurve 2 bzw. 2′ " " Leucin.
Kurve 3 bzw. 3′ " " Valin.
Kurve 4 bzw. 4′ " " Alanin.
Kurve 5 bzw. 5′ " " Glykokoll.
Kurve 6 (gestrichellte Linie, links) zeigt den Adsorptionsverlauf des Cl^-–Ions aus HCl–Lösung.
Kurve 6′(" , rechts) " Na^+–Ions " NaOH–Lösung.

Man sieht, dass Glykokoll und Alanin, wie schon Phelps und Peters (l. c.) nachgewiesen hagen, kaum adsorbiert werden, und zwar unabhängig

vom pH. Die Kurven für Valin, Leucin und Norleucin haben, wie aus der Figur ersichtlich, denselben Velaufstyps, indem jede einen Maximumerhebungsanteil zeigt, der sich über ein ziemlich weites Gebiet, welches den isoelektrischen Punkt (pHI*) einschliesst, erstreckt. Es wäre von Interesse, derartigen Adsorptionsverlauf mit Hilfe der Gleichung

$$\rho = \frac{1}{1 + \frac{k_a}{[H^+]} + \frac{k_b}{k_w}[H^+]} \qquad (1)$$

näher zu erörtern.

Hierin bedeuten:

ρ den Dissoziationsrest, k_a und k_b die Säure- und Basen-dissoziationskonstante der Aminosäure und k_w die Dissoziationskonstante des Wassers.

Als Beispiel hierfür führen wir Norleucin an. Die Ergebnisse der Versuche mit demselben sind ih Tabelle 1 zahlenmässig wiedergegeben.

Tabelle 1.

Adsorption aus den Lösungen: 0.05 m. Norleucin + c-Mol HCl bzw. NaOH. t = 18°C.

HCl bzw. NaOH c-Mol/L.	pH		Konzentration von Norleucin nach d. Ads. C_N in Mol. i. L.	Adsorbierte Menge in Millimol/g. Kohle.	
	vor d. Ads.	nach d. Ads.		Norleucin	Cl⁻ bzw. Na⁺
0.15 HCl	1.13	1.15	0.0276	0.448	0.547 Cl⁻
0.1	1.36	1.39	0.0273	0.454	0.492
0.075	1.49	1.50	0.0281	0.438	0.424
0.05	1.93	1.94	0.0250	0.499	0.354
0.025	2.46	2.47	0.0228	0.543	0.225
0.0125	2.91	2.97	0.0221	0.557	0.112
0.00625	3.23	3.39	0.0214	0.571	——
0	6.32	4.93	0.0213	0.574	——
0.00625 NaOH	9.18	8.89	0.0213	0.573	——
0.0125	9.48	**9.61**	0.0234	0.531	0.062 Na⁺
0.025	9.99	**10.21**	0.0258	0.484	0.120
0.045	10.93	**11.13**	0.0326	0.348	0.236
0.05	11.31	11.23	0.0333	0.333	0.260
0.06	12.16	11.94	0.0351	0.298	0.302
0.075	12.54	12.38	0.0353	0.294	0.332
0.1	12.79	12.71	0.0354	0.292	0.389
0.15	13.06	13.04	0.0351	0.298	0.433

Die PHI der drei angeführten Aminosäuren sind für 25°C sämtlich gleich etwa 6 (P. L. Kirk und C. L. A. Schmidt, J. Biol. Chem. **81**, 237, 1929; vgl. ferner Wo. Pauli und E. Valkó, Elektrochemie d. Kolloide, S. 376). Für 18°C dürften sie nicht weit verschieden von 6 sein. z. B. wurde für Norleucin aus Daten in Tab. 1 berechnet zu $k_a = 1.06 \times 10^{-10}$ und zu $k_b = 1.77 \times 10^{-12}$. Für k_w bei 18°C mit dem Wert $10^{-14.13}$ gerechnet, erhalten wir $pHI = 1/2\ (pk_a + pk_w - pk_b) = 6.17$.

Aus den Zahlenwerten für pH (3. Spalte) und für die Konzentration C_N von Norleucin (4. Spalte), beide bezogen auf die Gleichgewichtslösungen, in der Tabelle 1 können wir nach der Gleichung (1) die Konzentration C_n $(=\rho C_N)$ der elektrisch neutralen Norleucinmoleküle in der Gleichgewichtslösung berechnen.

Diese in die Gleichung

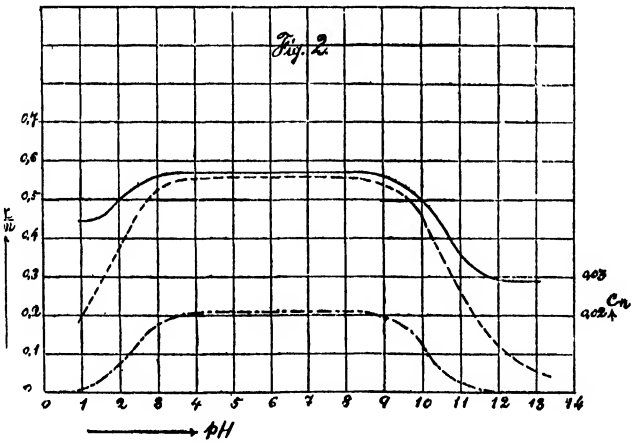
$$x/m = 2.27\ C^{0.364} \qquad (2)$$

an Stelle von C eingesetzt, erhalten wir die Grösse x_n/m, die annäherderweise die adsorbierte Menge des neutralen Norleucinmoleküls bei betreffendem pH angeben wird.

Die Adsorptionsformel (2) wurde allerdings aus den Resultaten der Adsorptionsversuche mit rein wässerigen Norleucinlösungen ermittelt, wobei als Adsorbens dieselbe Kohle wie in der vorstehenden Untersuchung verwendet worden war.

Da Norleucin mit seinen recht kleinen k_a– und k_b–Werten in rein wässeriger Lösung fast ausschliesslich als elektrisch neutrale Moleküle vorhanden ist, so sind wir imstande die Gleichung (2) als eine Adsorptionsformel für diese anzusehen.

Wir sind gewiss darüber klar, dass diese Gleichung auf dem obwaltenden Problem deshalb nur bedingt anwendbar ist, weil man hier in der Lösung die neben neutralen Norleucinmolekülen vorhandenen anderen Molekülarten (bzw. Ionenarten), die ebenfalls adsorbierbar und den Wechselwirkungen mit den ersteren zugänglich sein dürften, mit in Betracht ziehen muss. Vielmehr dürfte sie nur eine näherungsweise Mitteilung der adsorbierten Menge des neutralen Norleucinmoleküls gestatten.



In Fig. 2 ist x_n/m–pH–Kurve (gestrichellte Linie) neben der experimental ermittelten Adsorptionskurve (ausgezogene Linie) wiedergegeben, gleichzeitig

ist C_n–pH–Kurve (strichpunktierte Linie) mit eingezeichnet. Man sieht, dass die beiden erstgenannten Kurven im Gebiete der Maximumerhebung nahezu zusammenfallen. Hieraus folgt, dass Norleucin in diesem Gebiete (pH = etwa 3 bis 10) vorwiegend als neutrale Moleküle adsorbiert wird. Ausserhalb dieses Gebietes gehen die beiden Kurven mit ab- bzw. zunehmendem pH immer weiter voneinander ab. Unter Berücksichtigung der gleichzeitig erhöhten Adsorption von Cl^-– bzw. Na^+–Ionen (s. Fig. 1, Kurve 1 und 1') dürfen wir folgern, dass Norleucin auch als Kationen oder Anionen adsorbiert werden kann. Dass die beiden Endteilen der Adsorptionskurve zur Abszisse parallel verlaufen, könnte dadurch gedeutet werden, dass hier Norleucin vorwiegend als Kationen oder Anionen, deren Konzentration in der Gleichgewichtslösungen infolge der Anwesenheit von hinreichend grossem Überschuss von H^+– bzw. OH^-–Ionen nahezu konstant bleibt, adsorbiert werden.

Ferner geht aus Fig. 1 hervor, dass die Adsorption von Cl^-– bzw. Na^+–Ionen um so mehr begünstigt wird, je stärker die gleichzeitig in der Lösung vorhandene Aminosäure adsorbierbar ist. Obwohl sie an und für sich wenig kapillaraktiv sind, werden sie von den stärker adsorbierbaren H^+– bzw. OH^-–Ionen, und vor allem von den kapillaraktiven Aminosäureionen mit an die Oberfläche der Kohle gezogen.

Zusammenfassend können wir schliessen, dass die sämtlichen untersuchten oberflächenaktiven Aminosäuren am stärksten in einem ziemlich weiten den isoelektrischen Punkt einschliessenden Gebiet durch kohle aufgenommen werden, und zwar fast ausschliesslich als elektrisch neutrale Moleküle. Im stark sauren oder alkalischen Gebiet werden sie hauptsächlich als Kationen oder als Anionen adsorbiert.

Es besteht für die Adsorbierbarkeiten des neutralen Moleküls, des Kations und des Anions einer oberflächenaktiven Aminosäure die folgende Reihenfolge:

$$H_2N{\cdot}R{\cdot}COOH \text{ (oder vielleicht richtiger } H_3^+N{\cdot}R{\cdot}COO^-) > H_3^+N{\cdot}R{\cdot}COOH > H_2N{\cdot}R{\cdot}COO^-.$$

Hier fehlt also der gewöhnlich beobachtete Parallelismus zwischen der Oberflächenaktivität und der Adsorbierbarkeit an Kohle (vgl. III. Mitt.).

Hingegen findet dieselbe Regelmässigkeit wie bei der Oberflächenaktität auch hier statt, wenn bei einem bestimmten pH die Adsorbierbarkeiten der einzelnen Aminomonocarbonsäuren miteinander verglichen werden.

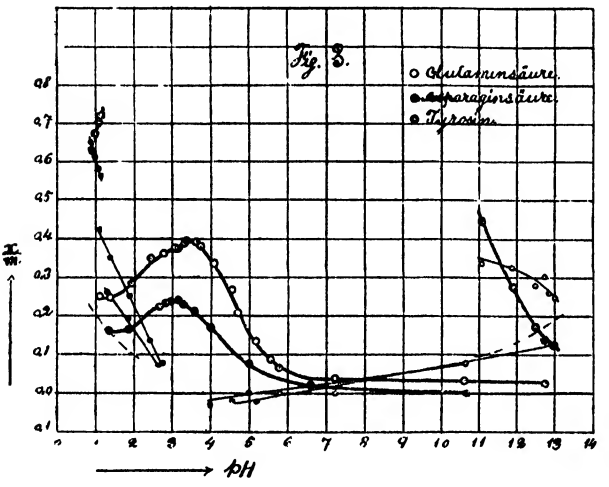
Die Reihenfolge der Adsorbierbarkeit lautet:

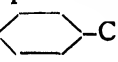
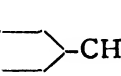
Norleucin > Leucin > Valin > Alanin > Glykokoll.

Schliesslich sei noch darauf hingewiesen, dass die Auffassung, derzufolge eine Pufferlösung sich bei Behandlung mit Kohle, infolge deren Ampholytoidnatur, der Neutralität nähern soll, nicht immer zutreffend ist (vgl. L. Michaelis: Die Wasserstoffionenkonzentration I, 2. Aufl., S. 209, 1922). Denn,

obwohl diese freilich in den meisten untersuchten Fällen als zutreffend bestätigt worden ist, doch konnten wir feststellen, dass alkalische Norleucinlösungen bei gewissen pH, wie aus Tabelle 1 ersichtlich, durch Zusammenbringen mit Kohle mehr alkalisch werden. Ähnliches wurde auch bei Leucin beobachtet.

2. Asparagin- und Glutaminsäure sollen nach Phelps und Peters (l. c.) durch das gereinigte Norit nur wenig adsorbiert werden, wobei sie eine Tendenz zu einer Minimumadsorption bei pH=etwa 5 aufweisen. Aus unseren Versuchen ergibt sich aber, dass sie, wie aus Fig. 3 ersichtlich, eine deutliche Maximumadsorption im pHI zeigen. Übrigens werden sie als Anionen kaum adsorbiert.



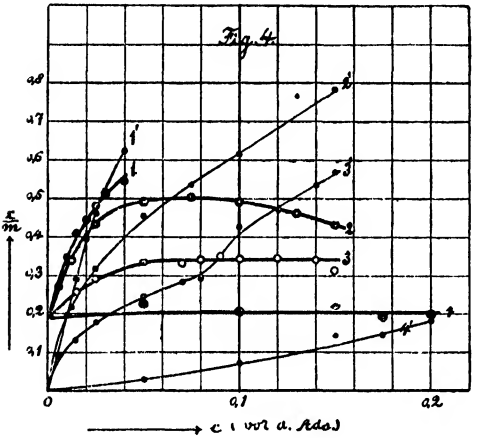
An Tyrosin sind die Versuche, wegen seiner geringen Löslichkeit, nur beschränkt ausgeführt worden. Indessen deuten die Verläufe der Kurvenstücken für Tyrosin in Fig. 3 darauf hin, dass es auch im pHI am stärksten adsorbiert werde. Auffallend ist, dass die Adsorptionskurve für Na^+-Ion bei Anwesenheit von Tyrosin, im Gegensatz zu den übrigen Fällen, mit zunehmendem pH abfällt. Dies dürfte darauf zurückzuführen sein, dass einwertiges HO-  -CH_2-$CH(COO^-)$-NH_2 erheblich stärker adsorbierbar ist als zweiwertig ^-O-  -CH_2-$CH(COO^-)$-NH_2, welches letztere, wie aus auf Grund der von Kirk und Schmidt (l. c.) angegebenen Daten durchgeführten Berechnungen hervorgeht, bei zunehmendem pH auf Kosten des ersteren zunimmt und bei z. B. pH=13 den fast ganzen Anteil des in der Lösung vorhandenen Tyrosins ausmacht.

3. Wenn eine HCl-Lösung von konstant gehaltener Konzentration mit wachsender Menge Aminosäure versetzt werden wird, so wird die H^+-Ionenkonzentration der Lösung in dem Masse abnehmen, als die Menge des Aminosäurekations zunimmt, die bei hinreichend grossem Zusatz der Aminosäure einem konstant bleibenden Endwerte zustreben wird. Dabei wird die Cl^--Ionenkonzentration stets nahezu konstant bleiben. Ähnliche Betrachtung lässt sich auch für den Fall anstellen, wo einer NaOH-Lösung steigende Menge

Aminosäure hinzugefügt wird.

Wenn nun die an sich schwach adsorbierbaren Cl^-– bzw. Na^+–Ionen von den stärker adsorbierbaren Aminosäureionen und H^+– bzw. OH^-–Ionen an die Kohlenoberfläche mitgeschleppt werden sollen, so liegt es nahe, zu vermuten, dass die Adsorption der ersteren aus den oben erwähnten Lösungen ab– oder zunehmen werde, je nachdem die betreffenden Aminosäureionen schwächer oder stärker adsorbierbar sind.

Fig. 4 gibt die Resultate der Adsorptionsversuche mit 0.1 *N.* HCl bei steigendem Zusatz von verschiedenen Aminosäuren. Als Ordinaten sind die adsorbierten Mengen in Millimol/g Kohle aufgetragen, als Abszissen die Konzentrationen C der zugesetzten Aminosäuren vor der Adsorption.



Hier stellen die dick ausgezogenen Linien die Adsorptionskurven für Cl^-–Ion bei Anwesenheit von Tyrosin (1), Norleucin (2), Glutaminsäure (3) oder Alanin (4) dar, die anderen die für die betreffenden Aminosäuren. Man sieht, dass die Cl^-–Adsorption durch die Anwesenheit von stark adsorbierbarem Tyrosin erheblich gesteigert wird. Auffallend ist, dass die Cl^-–Kurve bei Anwesenheit von Norleucin durch ein Maximum geht, um dann wieder allmählich abzufallen. Man könnte sich den Vorgang so vorstellen, dass die vermehrte Zugabe von Norleucin anfangs immer neues Norleucinkation auf Kosten des H^+–Ions in der Lösung schafft und somit zunehmende Adsorption des ersteren und abnehmende Adsorption des letzteren hervorbringt. Da die Adsorbierbarkeit des Norleucinkations die des H^+–Ions weit übertrifft, so nimmt die durch die beiden bedingte Cl^-–Adsorption stetig zu. Setzen wir nun nach erreichtem Maximum der Cl^-–Adsorption noch mehr Norleucin hinzu, so wird nunmehr die Adsorption von neutralen Norleucinmolekülen in immer steigendem Mass stattfinden. Diese werden die Norleucinkationen von der Kohlenoberfläche mehr oder weniger verdrängen und dadurch die Abnahme der Cl^-–Adsorption bedingen. Solche Verdrängungswirkung——über deren Mechanismus sei vorläufig dahingestellt——scheint bei weniger kapillaraktiver Glutaminsäure nicht ausgeprägt zu sein. Dass die Cl^-–Kurve (3) in Anwesenheit von Glutaminsäure nach merklichem Ansteigen zur Abszisse parallel verläuft, dürfte eher daraus hervorgehen, dass hier die Cl^-–Iononen bei steigendem pH ebenso stark abnehmend als HCl, wie zu-

nehmend als Glutaminsäureshydrochlorid adsorbiert werden.

Ferner ersieht man aus Fig. 4, dass sich das Schnittpunkt der Kurve für Cl^-–Ion und für die entsprechende Aminosäure mit steigendem Adsorbierbarkeit der letzteren nach kleineren C verschiebt, und zwar, mit Ausnahme des Falls von Alanin, auf der linken Seite des Äquivalenzpunktes (C=0.1). Es handelt sich hier also um hydrolytische Adsorption, die um so ausgeprägter ist, je stärker die betreffende Aminosäure adsorbierbar ist.

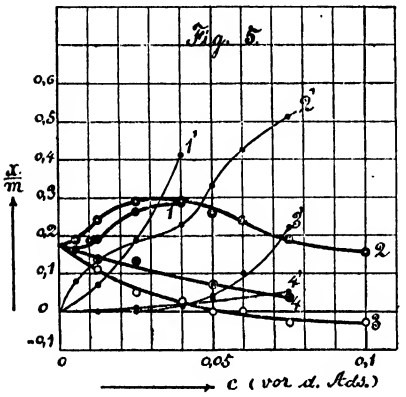


Fig. 5 stellt die Resultate der analogen Versuche mit 0.05 *N.* NaOH dar.

Die Adsorptionsverlaufe des Na^+–Ions bei Anwesenheit von Tyrosin (Kurve 1) oder Norleucin (Kurve 2) dürften sich in der ähnlichen Weise wie oben erklären lassen. Dass die Adsorptionskurven für Na^+–Ion bei steigendem Zusatz von Glutaminsäure (Kurve 3) oder Alanin (Kurve 4) abfallen, ist offenbar darauf zurückzuführen, dass die Natoriumsalze der genannten Aminosäuren nur sehr wenig adsorbierbar sind.

Studies on the Variations of the Constituents of Tobacco Plant During its Growth Periods.

Part I. Organic Constituents.

By

Hiroshi HASEGAWA and Sei IWATA.

(Received Feb. 2, 1932.)

Summary.

1. The purpose of the present investigation is to secure the basis for rational treatment in manuring and harvesting tobacco plants.

2. The material for this investigation is a negative sort of cultivated tobacco, called "Hatanoshu". The experiments were conducted through five growth periods with fourteen kinds of sampling, thus covering the whole developmental stages of the plant concerned, viz.

A. Seeds.

B. Seedlings (17 days after sowing and 10 days after germination.)

C. Young plant, suitable for transplanting (56 days after germination.)
D. Plant in the stage of topping (77 days after transplanting.)
   a. Leaves. b. Stem. c. Root. d. Flower stalk.
E. Mature plant, suitable for harvesting (103 days after transplanting.)
   a. Lower leaves (so-called "Doha" or "Lugs".)
   b. Sub-principal leaves (so-called "Chûha" or "Cutter".)
   c. Principal leaves (so-called "Hompa" or "Leaves".)
   d. Upper leaves (so-called "Tempa" or "Lips".)
   e. Stem.
   f. Root.
   g. Flower stalk and axillary buds.

3. Analytical determinations of the organic constituents were carried out after the methods of Kissling and also of Schlösing. The results were given for the following items: the weights of fresh and dry matter; the amounts of total-N, ammonia-N, nitric acid-N, amido-N, protein-N, nicotine-N, malic acid, citric acid, oxalic acid, acetic acid, tannin, pectinic matter, starch, glucose, succrose, dextrin, crude fibre and crude fat, each in percentages of fresh and dry weights of above mentioned samples. Calculations were also made for the content of these constituents in each one plant.

4. The most interesting result was obtained concerning the variation of nitrogenous compounds. Some peculiar features were also observed in the variations of such non-nitrogenous constituents as pectinic matter, organic acids, tannin, sugars, starch, dextrin and fibre.

On a Shark egg-oil.

By

Toyoki Ono.

(Received Feb. 15, 1932)

The Analysis of the egg-oil of Heptranchias deani Jordan and Starks was carried out.

Egg-oil was prepared from fresh by extraction with alcohol and ether, therefore contains somewhat phosphatides as lecithin. The general characteristics of oil are as follows:

Sp. gr. (at 30°C)	0.9220	Saponification value	171.26
Refract index (40°C)	1.4520	Reichert-Meissle value	2.9
Acid value	6.70	Acetyl value	21.08
Iodine value	144.43	Unsaponifiable matter	11.66%

Separation of mixed fatty acids.

Mixed acid were prepared from the soap solution of egg-oil by decomposition with H_2SO_4, and refined from ether solution: the separation of mixed acid into the saturated and unsaturated acids followed the Varrentrapp's method based on the different solubility for ether of both acid-lead salts. The yield was the former 21.4%, the latter 78%, the experimental loss 0.6%.

1. Saturated acids.

The composition of saturated acids was examined by the Heintz's fractional precipitation method of acid magnesium salt from alcohol: The combination-results obtained from three experiments are shown in the following table.

Fraction	Yield (g.)	m. pt. (°C)	Neutral Value		Mean mol. Weight
1	0.2810	56.5	206.9		271.2
2	0.5323	58.0	214.7	Mean Value 216.3	261.3
3	0.4431	57.5	217.8		257.5
4	0.4155	57.5	216.6		259.5
5	0.3674	58.0	218.1		261.8
6	0.3851	57.0	214.1		262.1
7	0.2143	56.5	212.1		264.6
8	0.2051	56.0	210.8		266.1
9	0.1260	55.5	209.0		268.4

This results show that the saturated acid consist mainly of the acid of melting point 57~58°C (*isopalmitic acid*), small amount of stearic acid and palmitic acid; the existence of iso-palmitic acid has been previously proved by many authors; my following experiment will prove its presence in analysed egg-oil:

Exp. (1) Shark-Solid acid (m. pt. 57.5)

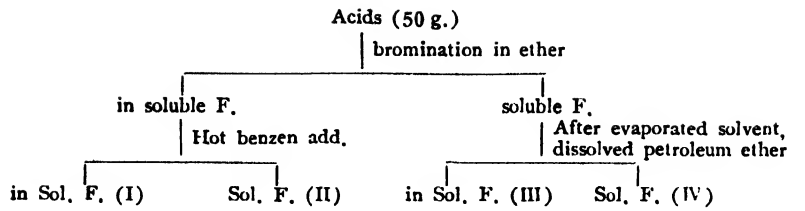
	1	2	3	4
m. pt.	58.0	57.5	57.5	57.5
	1	2	3	
m. pt.	57.5	57.5	57.5	

Exp. (2) Shark-solid acid (70%) + pure palmitic acid (30%) (m. pt. 56°)

	1	2	3	4
	59.0	58.5	57.5	55.0
	1	2	3	4
	59.5	58.0	57.5	56.0

2. Unsaturated acids.

The composition was determined as following system.



For each fraction of Br-fatty acids the experiment was taken as follows:

	Yield	Property	Found Br-content %	Calculated Br-content %
(I)	9.5	decomposed at 200°C	69.68	69.86 as $C_{18}H_{28}O_2Br_8$
(II)	4.0	m. pt. 178°C	64.03	63.32 as $C_{18}H_{30}O_2Br_6$
(III)	12.5	viscous	61.37	63.32 as $C_{18}H_{30}O_2Br_6$
(IV)	59.0	oily	35.58	36.18 as $C_{18}H_{34}O_2Br_2$

The original unsaturated acids of fraction (I), (II) and (IV) are respectively corresponded with Clupanodonic acid, Linoleic acid and Oleic acid; the fraction (III) absorbed some Iodin (Iodin value=4.35), so repeating the bromination, the obtained brom-compound showed 62.36 of Br-content, and changed so its property as melt at 40°C; I suppose the original acid of this Br-acid will be one new isomeric form of linoleic acid. On the ether soluble hexa-brom-compound of linoleic acid, as a result of their studing Erdmann and Bedford (1909), determined such a linoleic acid is β-form.

3. Unsaponifiable matter.

This substance gave a yellow-white liquid, Iodin value 62.6; dissolved in four volumes of acetone, at cold temparature separated into the crystaline and non-crystaline parts.

Crystaline part:-

The fractional crystalization from alcohol solution made the three compound:

Fraction	Yield (g.)	m. pt. (°C)	m. pt. of acetate	Sap. value of acetate
(I)	14.3	146	114	113.00
(II)	2.4	59	—	—
(III)	0.7	51	oily	—

(I) correspond with cholesterol, (II), (III) will be respectively octodecyl-and cetyl alcohol.

Liquid part:-

The acetate compounds prepared by addition of acetic anhydride were fractioned in several parts by dry-distillation under 24 mm. pressure:

Fraction	Temp.	Yield (g.)	Sap. value	Iodin value	Iod. val. of orginal state
(I)	250	7	263.71	67.9	80.6
(II)	250~270	6	248.90	61.8	75.3
(III)	Residue	0.5	128.50	—	—

The characteristics of fraction (I) conform to those of selacyl alcohol ($C_{21}H_{42}O_3$) discoverd by Tsujimoto & Toyama from many Shark-liver oils. With regard to fraction (II), I suppose it will be the one unknown unsaturated alcohol as C_{22}……, C_{24}……, or those combination compound.

Summary.

1. Fatty acids contain 21% of saturated acids, 79% of unsaturated acids: the saturated acids consist mostly of iso-palmitic acid, and small amount of stearic and palmitic acid.

2. The unsaturated acids consist of oleic acid (78%), linoleic acid (14%), and clupanodonic acid (6%); 80% of linoleic acid takes the one isomeric form, which hexa-form acid is soluble in ether.

3. Unsaponifiable matters contain cholesterol (40%), octodecyl alcohol (5.5%), cetyl alcohol (1.9%), selacyl alcohol (25%) and unknown alcohol (21%).

I wish to express my sincere gratitude to Prof. Dr. U. Suzuki and Prof. Dr. M. Yamakawa for the directions given me of this investigation.

On the Study of Nitrogen, Phosphorus and Potassium Absorption and Utilization of Rice Plant at Different Stages of Growth by Means of Water Culture.

By

Yoshiaki Ishizuka.

(Received March 4, 1932.)

Experiment was conducted to study the nitrogen, phosphorus and potassium absorption and utilization of rice plant at different stages of growth by means of water culture for economical fertilization.

The following conclusions were reached.

1) The growth of rice plant was not hindered by the lack of nitrogen in nutrient solution till the 2nd week after the beginning of growth, if there was a supply of nitrogen after that time. When the supply of nitrogen failed after the 3rd week, the growth was hindered in spite of the immediate supply

of nitrogen after that time, so the ripening was not complete. But the apparent color recovery was very striking.

The growth was not hindered by the lack of phosphorus till the 3rd week after the beginning of growth if there was a supply of the same after that time. When the supply of phosphorus failed after the 5th week the growth was hindered in spite of the immediate supply of the same after that time.

The growth was not hindered by the lack of potassium in nutrient solution after the 5th week from the beginning of the growth provided there was a supply of the same after that time. When the supply of potassium failed after the 7th week the growth was hindered or the plants died in spite of immediate supply of the same after that time.

2) The existence of nitrogen in nutrient solution was necessary for rice plant till the 7th week after beginning of growth. The existence of nitrogen till the 9th week was sufficient for its perfect growth. So its growth will not be hindered by the lack of nitrogen after that time.

The existence of phosphorus was necessary till the 5th week after beginning of growth. The existence of the same till the 7th week was sufficient for ist perfect growth. So its growth will not be hindered by the lack of phosphorus after that time.

The existence of potassium was absolutely necessary till the 9th week after beginning of growth. And furthermore the growth will be hindered by the lack of potassium after that time.

3) The degree of nitrogen and phosphorus utilization of rice plant was high at the earlier stages of growth and the later the first supply, the lower the degree of utilization. The maximum point of the degree of utilization was supposed to lie between the 3rd and 5th week after the beginning of growth.

In the case of potassium, though its existence was necessary through all stages of growth, the maximum utilization was supposed to be between the 4th and 5th week after the beginning of growth.

4) In the case of nitrogen fertilization of rice plant, it seemed to be expedient to supply nitrogen at the early stage of its growth so that it might absorb the total quantity of nitrogen, necessary for perfect growth, within 9 weeks.

In the case of phosphorus fertilization it seemed to be expedient to supply phosphorus at the early stages so that it might absorb the total quantity of phosphorus necessary for the growth within 7 weeks after beginning of growth.

In the case of potassium fertilization it seemed to be expedient to supply potassium throughout all stages of growth.

Studies on the Yeast Growth.

By

Takeo SHIMODAIRA.

(Received March 5, 1932.)

The problem of the yeast growth was treated in the present investigation. When the ammount of the pitching yeast in a certain fermenting solution is small, its yield calculated on the ratio to the pitching yeast cells, is considerably effected by the quantity of the initial cells. I have therefore adopted in my experiment relatively a large number of cells under which condition a slight difference between the quantities of the pitching yeast for a series of experiments insignificantly upon the ratio and the result can be undoubtedly determined.

I have undertaken at first to determine the most favorable concentration of sugar of Hayduck's solution and beer wort for the yeast growth and found 6～7% for the former and 15～16% for the latter.

Researches on the Electrolytic Reduction Potentials of Organic Compounds. Part XIII.

Reduction Potential of Azobenzene.

By

Masuzo SHIKATA and Isamu TACHI.

(*Agricultural Chemical Institute, College of Agriculture, Kyoto Imperial University.*)

(Received April 10, 1932.)

Summary.

(1) The electrolytic reduction potentials of azobenzene were measured by the polarographic method with the dropping mercury, stationary mercury and platinum cathode.

(2) From R. P. measured by the dropping cathode, the electrolytic reduction of azobenzene may be regarded as a reversible azohydrazo system in the range of 1.2 to 5.0 by pH.

(3) R. P. measured by the stationary mercury cathode with a cathodic surface area of 0.785 mm^2 gave the same value as that of the dropping

cathode.

(4) The polarograms obtained with the stationary mercury cathode has always maximum and this maximum was due to the accumulation of the reduction product on the cathode.

(5) R. P. measured by the platinum cathode with a surface area of 0.785 mm^2 were about 150~200 mV more negative than other two cases.

Researches on the Electrolytic Reduction Potentials of Organic Compounds. Part XIV.

Reduction Potentials of Dinitrophenols.

By

Masuzo SHIKATA and Nobushige HOZAKI.

(Agricultural Chemical Institute, College of Agriculture, Kyoto Imperial University.)

(Received 10, April 1932.)

Summary.

(1) The electrolytic reduction potentials of α-, β- and γ-dinitrophenols were measured by the polarographic method.

(2) The reduction of dinitrophenols took place in two stages as dinitrobenzenes due to their two nitro groups.

(3) The reducibilities of dinitrophenols are arranged in order of $\gamma > \beta > \alpha$ in the first stage and $\beta > \alpha > \gamma$ in the second stage.

(4) The comparison of the reduction potentials of dinitrophenols, dinitrobenzenes, nitrobenzene and nitrophenols was made and their mutual relation was found to obey our negativity rule.

(5) The solubilities of dinitrophenols by the polargraphic method at 25° C are as follows:

2,4 (or α)-dinitrophenol : 0.0865 g/L
2,6 (or β)-dinitrophenol : 0.2853 g/L
2,5 (or γ)-dinitrophenol : 0.1123 g/L

Researches on the Electoolytic Reduction Potentials of Organic Compounds. Part XV.

Reduction Potentials of Nitranilines.

By

Masuzo SHIKATA and Eiichi TAGUCHI.

(*Agricultural Chemical Institute, College of Agriculture, Kyoto Imperial University.*)

(Received April 10, 1932.)

Summary.

(1) The electrolytic reduction potentials (R. P.) of o–, m– and p–nitranilines were measured by the polarographic method.

(2) Since nitraniline is a weak base, the reduction in a suitable acidic solution took place in two stages, that is, the reductions of the dissociated and undissociated molecules.

(3) R. P. of dissociated molecules are more positive than those of undissociated molecules.

(4) On the reducibility of o–, m– and p–nitranilines, meta is more reducible than ortho and para. This may be due to alternating polarity of benzene ring.

(5) R. P. of ionic form of nitraniline is more reducible than nitrobenzene but R. P. of molecular form is less reducible than it, but m–derivative is an exception. We may suggest that NH_2 group is electropositive.

Chemical Researches on Bog-moss. Part I.

Chemical Composition of Sphagnum Fimbriatum, Wils.

By

Masuzo SHIKATA and Mamoru WATANABE.

(*At the Forest Products Laboratory, Depart. of Agric. Chemistry, Kyoto Imperial University.*)

(Received April 10, 1932.)

1. We determined the chemical composition of bog–moss which was produced at Karafuto Island, Japan.

2. Analytical method was briefly described, and the results of the analyses were discussed.

3. The bog-moss changed its colour by heating it in the thermostat at the temperature of 105°C.

4. The cellulose which was isolated by the usual chlorination method was partly carbonized in the same thermostat.

5. The amount of the cellulose separated by the usual chlorination method was far more than the residue of the alkali extract.

6. Therefore it is more advisable to apply the modified chlorination method, using 1% caustic soda solution in the determination of cellulose.

7. There was great difference in the amounts and the character of celluloses which were isolated by the above two methods.

8. We pointed out some different properties which were exhibited by moss and wood cellulose.

9. Lignin was also differed from that of wood in some respect.

10. The amount of alpha-cellulose was remarkably small. It is only 9 % against air dried sample.

Biochemistry of Filamentous Fungi. I.

Colouring Matters of Monascus Purpureus Went. Part I.

By

Hidejiro NISHIKAWA.

(Received March 28, 1932.)

Résumé.

1. From the mycelial felt of Monascus purpureus Went cultured in a synthetic medium a crystalline colouring matter, monascorubrin, $C_{29}H_{24}O_5$, melting at 136°, was isolated in red prisms or needles. Its dihydrocompound is reddish yellow triangular platelets. These two substances have exceedingly high laevo-rotatory power. A crystalline bromo-derivative of monascorubrin was also prepared.

2. A smaller fraction of monascorubrin is converted into a crystalline yellow colouring matter, monascoflavin, $C_{17}H_{22}O_4$, melting at 145°, when the former is treated in an alcoholic solution with hydrogen peroxide. Monascoflavin can also be obtained from aged mycelium of Monascus purpureus. It crystallises in rhombic platelets. Its dihydro- and dibromo-derivatives are crystalline, while its monoacetyl derivative can not so far be obtained in crystalline form. Contrary to monascorubrin and its dihydro-derivative, monascoflavin and its dihydro-compound are strongly dextro-rotatory. Di-

hydromonascoflavin has phenolic properties.

3. Neither monascorubrin nor monascoflavin contains methoxyl group.

4. Monascorubrin on potash fusion produces with very poor yield a mixture of lower fatty acids among which caproic acid is most abundant and identified through its anilide. From the non-volatile portion of acidified fused material ether extracts a small quantity of a substance giving purple $FeCl_3$ reaction.

5. Carbon skeleton of and structural relation between monascorubrin and monascoflavin are still obscure and their formulae are yet to be corroborated upon stronger experimental basis. So far the presence of a double bond, a C_6 straight chain, and presumably a benzene nucleus in the monascorubrin molecule may be inferred from above. Further investigations are going on.

On Soil-acidity and Electro-dialyzable Aluminium, Iron and Etc.

By

S. Osugi and M. Aoki.

(Received April 23, 1932.)

Résumé.

It is well known fact that potassium chloride extract of acid-soil contains aluminium and iron in the amount corresponding to the acidity and yet it is not determined whether these aluminium and iron are primary product of base-exchange or not.

In order to investigate this question, the present authors made some experiments and the results will be reported as follows.

At first, the authors experimented that soil-acidity is much increased after the treatment of soil with a dilute solution of $FeCl_3$, $AlCl_3$, $CuCl_2$ or $NiCl_2$ and that the increase is almost parallel to the concentration of hydrogen ion of the solution.

The above result shows that the hydrogen ion of the solution plays much important role upon the increase of acidity.

The result of the electro-dialysis of the treated soil shows that the speed of extraction of aluminium and iron in the soil differs much from that of absorbed base and that most part of aluminium and iron dialyzed, deposits in the menbrane.

The behavior of aluminium hydroxide and ferric hydroxide added to soil

in the electro-dialysis, is similar to that in the treated soil.

The dialysis of acid-soil shows also similar result.

From these results, the authors may conclude that the state of aluminium and iron in acid-soil and in the treated soil is not same as absorbed bases and that they move as colloidal particles in electro-dialysis.

In the case of the soil treated with copper chloride or nickel chloride solution, most part of copper and nickel absorbed, is electro-dialyzed and the speed of dialysis is similar to that of absorbed base.

The authors found at the same time a great difference between these two cases, that is, in the former case (alminium and iron), most part of metal dialyzed, deposits in the menbrane and in the latter (absorbed base), the base is extracted in the cathode solution.

The authors conducted one more experiment to test the effect of anode acid solution upon the extraction of metal in the cathode solution by using two chambers (with anode solution) and three chambers cell (without anode solution) and found a distinct effect of anode solution and also greater effect with soil of stronger acidity and with metal of greater solubility in acid solution.

From these experimental results, the authors may conclude that in electro-dialysis, alminium and iron in soil move primarily as colloidal particles and deposit in the menbrane and only a small portion of them is extracted in cathode solution as a secondary product between colloid and anode acid solution.

On the Nitrogenous Constituents of Rice-Vinegar.

By

Kotaro NISHIDA.

(*Kagoshima Agricultural College, Kagoshima, Japan.*)

(Received May 18, 1932.)

The rice-vinegar experimented with was prepared at Fukuyama, Kagoshima prefecture; and the general composition of the samples is as follows:-

	Sample (A)	Sample (B)
Specific gravity	1.0291	1.0248
	g. in 100 c.c.	
Crude protein	1.3706	1.4369
Protein	0.1127	—

Extract	4.5618	3.8344
Total acid (as acetic acid)	6.1172	5.7310
Volatile acid (as acetic acid)	5.4732	——
Non-volatile acid (as succinic acid)	0.6301	
Reducing sugar (as glucose)	0.4200	
Ash	1.3210	1.0572
Fe_2O_3	0.0344	0.0341
P_2O_5	0.0794	0.0627

The various forms of nitrogen in these samples were also determined:—

	Sample (A)						Sample (B)	
	Total N	Protein N	Non-Protein N				Total N	Ammonia N
			Total N	Ammonia N	Organic base N	Other N		
g. in 100 c.c.	0.2193	0.0180	0.2013	0.0285	0.0683	0.1045	0.2299	0.0225
Ratio (Total N as 100)	100.0	8.2	91.8	13.0	31.1	47.7	100.0	9.8

Isolation and Identification of Organic Bases.

For the isolation of the organic bases 8 litres of the sample (B) were evaporated under reduced pressure to a small volume, almost equal to 1/4 of the original. After this operation the protein substance and other impurities were removed by lead acetate, and according to the general method researches were made about the organic bases, fractionally precipitated by phosphotungstic acid.

Phosphotungstic acid-precipitate I.

The first formed precipitate by phosphotungstic acid was fractionated into three fractions.

(1) PURINE BASE–FRACTION ($AgNO_3$–Precipitate):—The yield of the base from this fraction was 0.40 g. as hydrochloride. Its picrate crystallised in characteristic hairly needles; greenish yellow, sparingly soluble in water, decomposed at 280~281°C (uncorr.). The chloroaurate of the base formed golden yellow prisms, melted at 261°C (uncorr.) with decomposition.

0.1233 g. Subst. 0.0589 g. Au 47.77% Au

Calc. for Adeninechloroaurate ($C_5H_5N_5 \cdot 2HCl \cdot 2AuCl_3 \cdot H_2O$) 47.35% Au

(2) ARGININE–FRACTION ($AgNO_3$ & $Ba(OH)_2$–Precipitate):—The syrupy hydrochloride of the base obtained from this fraction was dissolved in cold absolute alcohol, and precipitated with saturated alcoholic solution of $HgCl_2$. The yield of the base from the $HgCl_2$–precipitate was 0.40 g. as hydrochloride. Its picrate formed greenish yellow prisms, hardly soluble in water, melted at 252°C (uncorr.) with decomposition. The chloroaurate of the base formed

yellow prisms, decomposed at 233°C (uncorr.)

0.2382 g. Subst.	0.1215 g. Au	51.01% Au
Calc. for Putrescinechloroaurate ($C_4H_{12}N_2 \cdot 2HCl \cdot 2AuCl_3$)		51.35% Au

(3) LYSINE-FRACTION (Filtrate from $AgNO_3$ & $Ba(OH)_2$-Precipitate):—The hydrochloride, which was freed from water obtained by this fraction, was treated with cold absolute alcohol and separated as follows.

(a) Insoluble portion by cold absolute alcohol:—Yield; 0.80 g. Its picrate formed yellow prisms, decomposed at 250~251°C (uncorr.). The chloroplatinate of the base crystallised in golden yellow thin plates, and gave the following analysis.

0.1230 g. Subst.	0.0486 g. Pt	39.51% Pt
0.1102 g. Subst.	0.0429 g. Pt	38.93% Pt
Calc. for putrescinechloroplatinate ($C_4H_{12}N_2 \cdot 2HCl \cdot PtCl_4$)		39.13% Pt

The above results agree fairly with putrescine derivatives.

(b) Dissolved portion by cold absolute alcohol:—Saturated alcoholic solution of $HgCl_2$ was added to this portion, and 0.20 g. of the hydrochloride of the base from the $HgCl_2$-precipitate was obtained. Its picrate forms greenish yellow prisms, melted at 253°C (uncorr.) with decomposition. The chloroplatinate of the base was prepared in golden yellow thin plates.

0.2129 g. Subst.	0.0830 g. Pt	38.99% Pt
Calc. for Putrescinechloroplatinate ($C_4H_{12}N_2 \cdot 2HCl \cdot PtCl_4$)		39.13% Pt

Phosphotungstic acid-precipitate II.

The hydrochloride of the base freed from water obtained by this precipitate was treated with cold absolute alcohol and separated into two portions.

(1) INSOLUBLE PORTION BY COLD ABSOLUTE ALCOHOL:—Yield; 0.60 g. Its picrate forms light yellow prisms, hardly soluble in water, decomposed at 252~253°C (uncorr.). The chloroaurate of the base forms yellow prisms, hardly soluble in water, melted at 234°C (uncorr.) with decomposition.

0.3230 g. Subst.	0.1657 g. Au	51.30% Au
0.2780 g. Subst.	0.1422 g. Au	51.15% Au
Calc. for Putrescinechloroaurate ($C_4H_{12}N_2 \cdot 2HCl \cdot 2AuCl_3$)		51.35% Au

(2) DISSOLVED PORTION BY COLD ABSOLUTE ALCOHOL:—Saturated alcoholic solution of $HgCl_2$ was added to this portion and separated as follows.

(a) $HgCl_2$-Precipitate:—From the precipitate of $HgCl_2$, 0.20 g. of putrescinehydrochloride was crystallized out. Its picrate forms greenish yellow prisms, hardly soluble in water, decomposed at 252~253°C (uncorr.). The chloroaurate crystallised in yellow prisms, hardly soluble in water, decomposed at 233°C (uncorr.).

0.3370 g. Subst.	0.1732 g. Au	51.39% Au
Calc. for Putrescinechloroaurate ($C_4H_{12}N_2 \cdot 2HCl \cdot 2AuCl_3$)		51.35% Au

(b) Filtrate from $HgCl_2$-precipitate:—0.10 g. hydrochloride of the base

was obtained from this portion. Its picrate forms yellow prisms, melted at 202°C (uncorr.). The chloroplatinate forms yellow thin plates, easily soluble in water, decomposed at 226°C (uncorr.).

0.0241 g. Subst.	0.0086 g. Pt	35.68% Pt
Calc. for Ornithinechloroplatinate ($C_5H_{12}N_2O_2 \cdot 2HCl \cdot PtCl_4$)		35.95% Pt

Summary.

The yield of the nitrogenous compounds, isolated from 8 litres of rice-vinegar, is as follows.

Adenine (as hydrochloride)	0.40 g.	Putrescine (as hydrochloride)	2.20 g.
Ornithine (")	0.10 g.	Ammonia	2.19 g.

On the Changes of Cyanamide in the Soil. II.

The Decomposition of Dicyanodiamide and Guanylurea in Paddy-soil.

By

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(Received May 24, 1932.)

I. Introduction.

A previous investigation by the author (Nippon Nogeikwagaku Kwaishi and this Bulletin, 1930) showed that the formation of ammonia from cyanamide, mainly through urea, is almost same under both soil conditions, namely dry-field (normal soil) and paddy-field (water-logged soil). However, there is a noted difference in the water-logged soil when dicyanodiamide is transformed from cyanamide. Under the water-logged soil condition dicyanodiamide is comparatively easily ammonified, especially in the summer when the soil temperature is relatively high. The author has proved that this is the main reason for the special fertilizing value of dicyanodiamide upon the paddy-rice, in contrast to its harmful effect on ordinary farm crops.

Further studies on the decomposition of dicyanodiamide and its related compound, guanylurea, in water-logged soil have been carried out by the author, with the object of solving some of the problems regarding the unusual decomposion of nitrogenous compounds in paddy-soil.

II. Influence of soil types, temperature, and soil treatment upon dicyanodiamide-decomposition in water-logged soil

(with 5 tables in original paper).

1. 7 different soil samples from paddy-fields and dry-fields were tested under the water-logged condition, at the incubation temperature of 30～36°C; the ammonification of dicyanodiamide in above condition is a general phenomenon, influence of different soil types upon the dicyanodiamide decomposition is not distinct, but rather complicated, and largely depends on the soil treatment.

2. The optimum temperature for the ammonification of dicyanodiamide in water-logged soil is 35～45°C. In Kagoshima section, during July and August, the temperature above 30°C prevail in the surface water and the soil surface of paddy-fields, and on cloudless days the temperature of the surface water occasionally reaches 40°C, hence the temperature that may be regarded as favorable for dicyanodiamide decomposition seems to be frequently attained in the paddy-soil during paddy-rice cultivation.

3. Air drying of the soil, whether exposed to direct sunlight under natural conditions, or in the diffused light of the laboratory, markedly increases the ammonifying power of the soil in a water-logged state, both for the soil organic nitrogen and the added dicyanodiamide. For example, the rate of ammonia-formation from dicyanodiamide in the water-logged condition by an air-dried soil was two times more than that by the fresh soil, being 10 mg. nitrogen per 100 g. dry soil in the former case against 4 mg. nitrogen in the latter, at the end of 6 weeks with an incubation temperature of 30～36°C. The emmission of a putrid smell always took place when the air-dried soils were incubated in a water-logged condition.

4. A preparatory treatment of the soil with toluene or ether, or the addition of 0.25% mercuric chloride to the water-logged soil, nearly stops the ammonification of dicyanodiamide in the water-logged condition, at 25～28°C. The presence of 0.125% mercuric chloride in the water-logged soil, however, does not completely suppresses this action at the same temperature.

III. Mechanism of the decomposition of dicyanodiamide (with 10 tables).

1. The formation of guanylurea from dicyanodiamide was noticed when the soil was partially sterilized. From this fact it seems that, in the course of the ammonification of dicyanodiamide, at least some portion of the dicyanodiamide would be converted to guanylurea as an intermediate product. The conversion of dicyanodiamide to guanylurea is probably brought about by a purely chemical (or physico-chemical) process, such as hydrolysis, but not by living micro-organisms, while the formation of ammonia from guanylurea in water-logged soil may be due to the agency of active micro-organisms. Whether, however, the intermediate production of guanylurea from dicyano-

diamide by nonmicrobial agency is the decisive factor for the ammonification of dicyanodiamide in water-logged soil, or the direct ammonification of dicyanodiamide by microbial activity is the main process of dicyanodiamide-decomposition, is not yet clear. The affirmation of this question needs further investigation.

2. Guanylurea is well absorbed by the soil, but the large parts of guanylurea in the soil are leached by the author's method of soil extraction, the method being the treatment of the soil with ferric sulphate solution and lime powder previous to the filtration.

3. The separate determination of dicyanodiamide and guanylurea in the soil may be accomplished by the treatment of the soil sample with copper sulphate solution etc., as dicyanodiamide alone appears in the filtrate, and guanylurea remains in the soil residue.

4. A marked difference in the rate of ammonification of guanylurea between normal soil and water-logged soil is noted, as in the case of dicyanodiamide. Guanylurea, examined in the forms of sulphate and phosphate, is very slowly ammonified in the normal soil condition, as already pointed out by Jacob and others (J. Agr. Res., 1924.), while it is relatively rapidly decomposed in water-logged soil, its ammonification being more rapid than that of dicyanodiamide.

5. It is of interest to find the variation of dicyanodiamide decomposing power by the same paddy-field soil in different season of the year. The soil, which was taken in February from a paddy-field under fallowing in the normal soil condition, ammonified only 5 mg. of dicyanodiamide nitrogen per 100 g. dry soil within 30 days at incubation temperature of 26～28°C when it was brought to the water-logged condition immediately after it was taken from the field, while, in September, when the field was in the later stage of paddy-rice cultivation (It was maintained in a flooded condition for nearly 3 months) the same soil ammonified 8.5 mg. of dicyanodiamide nitrogen per 100 g. of dry soil within 21 days at the same incubation temperature. This difference in the dicyanodiamide-decomposing power may be more largely due to the effect of the soil aeration or the water-logging to which the paddy-field was subjected and less to the mere seasonal difference. The "Kan-den", that is the paddy-field having good drainage, is usually flooded only during the season of paddy-rice cultivation (that is, from early summer to autumn), and after that time (from fall to spring), the field is in the normal soil condition. In the laboratory trials, similar results were obtained with regard to the influence of soil aeration and water-logging upon the dicyanodiamide-decomposing power of a soil. A soil kept in normal soil condition for a month has shown a strong retarding tendency for the dicyanodiamide-decomposition

in the water-logged condition. The longer the period during which the soil was subjected to water-logging, the more the dicyanodiamide-decomposing power was increased.

6. The relations of soil cultivation system and soil treatment to the ammonification of guanylurea in water-logged soil are quite identical to that of dicyanodiamide decomposition. Comparing two soil samples, taken in September from different plots in the same field (the normal soil plot under buckwheat and the paddy-soil plot under paddy-rice), a marked difference was observed in the rate of guanylurea-ammonification in the two soils. With the addition of 10 mg. guanylurea-nitrogen per 100 g. dry soil the rate of ammonification was only 2.6 mg. by the normal soil against 8.3 mg. by the paddy-soil (both in a water-logged condition) after 20 days incubation at 26 ~28°C. A more striking reduction in the guanylurea-ammonifying power in the water-logged condition was observed when the paddy-soil sample was stored under normal soil condition (at a moisture content of one-half saturation) for 2 months, in a wide-mouthed bottle with cotton plug, in the laboratory. The stored samples had a tendency to grow more acid and the pH value decreased from 6.0 to 4.7 after 2 months, when it was brought to a water-logged condition and incubated at 26~28°C, it did not show the guanylurea-ammonifying power at all even after 30 days.

From these facts, it may be said that, the prolongation of a liberal aerobic condition in the normal soil state is detrimental to the agency for the guanylurea-ammonification process. On the arable soil of the ordinary farm, however, the soil condition that may be regarded as harmful for the guanylurea-ammonifying agency will not be present over long periods but will be effected by frequent rainfalls and temporary drying of the surface soil, etc.. Consequently, most soils always show, to some degree, the guanylurea-ammonifying power, when the soils are brought to a water-logged condition.

7. The guanylurea-decomposing power of the soil, which was weakened or almost lost by subjecting the soil to aerobic, normal, soil conditions, was gradually restored by the addition of calcium carbonate when the soil was water-soaked, thus increasing the decreased pH value of the soil; where the addition of 0.5 g. calcium carbonate per 100 g. dry soil was made, the pH value of the soil quickly lose from 4.7 to 6.2, and to 7.3 after 30 days incubation. The weakened guanylurea-ammonifying power of the soil was, also, restored without increasing the pH value, but simply inoculating the soil with the paddy-soil (5 g./100 g.), which was kept in the water-logged condition for a long time.

From above results, it can be assumed that the improvement of the guanylurea-ammonifying power of the soil should not be ascribed merely to

the modification of the pH value of the soil, but may be explained by the oxidation–reduction potentials of the soil. Under an environmental condition of a high oxygen tention, where pH is markedly raised, the partial sterilization of soil seems to take place, thus destroying certain species of the guanylurea–ammonifying micro–organisms.

IV. Conclusion.

In the majority of water–logged soils, dicyanodiamide is ammonified at the usual summer temperature.

In the course of the ammonification of dicyanodiamide, at least some portion of dicyanodiamide is converted into guanylurea as an intermediate decomposition product.

The environmental conditions, under which the soil is subjected prior to the incubation of water–logged state, greatly affect the ammonification of dicyanodiamide and guanylurea. In an environment of a high oxygen tension, the partial sterilization of soil seems to take place and destroys certain species of dicyanodiamide and guanylurea–ammonifying micro–organisms.

On the Natural Pigments of Raw Silk Fibre of the Domestic Cocoon. (Part IV).

Carotin and Xanthophyllesters.

By

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(Received May 4, 1932.)

The natural yellow pigments of the domestic cocoons were hitherto identified by the author as chiefly consisted of xanthophylls, from which crystallizable pigment was isolated and identified as lutein (leaf xanthophyll).

In this research the occurrence of other natural yellow pigments such as carotin and xanthophyllesters, which might be possible as in the case of xanthophyll to be derived from the mulberry leaves, is searched and attained the following results.

1) Xanthophyllesters such as physalien, Helenien do not exist in the yellow cocoons neither in golden yellow nor in orange yellow species, though the occurrence of xanthophylls in the mulberry leaves may be assumed in a part as such esters.

2) The fact, that carotin of the yellow cocoons does not exist in the pure crystallizable but partially oxidized state, is verified by the spectrometric method, using as control the purely crystallized carotin of carrots and its partially oxidized one.

3) The carotin content of the yellow cocoons shows 0.6～0.8 mg. per 100 g. air dried cocoon layers: these figures show from one-fortieth to one-seventieth of the xanthophyll content.

4) As the carotin fractions of both golden yellow and orange yellow cocoons are the same regarding their coloring tones in appearance and their modes of absorption bands of the visible part, different pigments can't be supposed to exist in this fraction. The origin of the different coloring tones of both species must be asked in another fractions.

On the Nutritive Value of Pentosan. III.

The Glycogen Accumulation in the Body of Rats by the Xylan Feeding.

By

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(Received May 30, 1932.)

In a previous communication of this investigation[2] it was concluded that the total amount of glycogen accumulated in the body of rabbit, when xylan was administered, is nearly equal to that accumulated by the administration of the same amount of starch. In this paper a brief report is made on the effect of xylan feeding upon the glycogen content in the albino rat, which were selected as an example of omnivorous animals.

Experimental.

Young albino rats were divided into three groups, one of them was subjected to a fasting test, the other to a xylan feeding test, and the latter to a starch feeding test as a collateral. The detail of the experimental procedure was nearly equal as described in the second report of this investigation. The results obtained in ragard to both the glycogen content and blood constituents, which were also studied in this connection, may be shown in Table I, II, III.

Table I. The fasting test.

Date. (1930~1932)	'30. Oct. 1	Oct. 28	Oct. 31	'31. Jan. 29	Jan. 30
Sex of albino rat.	male	male	female	female	female
Hours fasted.	25.5	24.0	24.0	24.5	24.0
Body weight, g.	208	180	105	133	149
Liver weight, g.	8.4	10.5	4.5	7.0	7.8
Liver glycogen, mg. in 100 mg.	11	37	32	60	41
Muscle weight (calculated), g.	97.8	84.6	49.4	62.5	70.0
Muscle glycogen, mg. in 100 g.	21	25	105	274	118
Total glycogen, in 100 g. body.	10.3	14.0	50.6	132.0	57.6
Do. average.	12.2		80.1		
Total blood sugar, mg. in 100 c.c.	—	—	106	96	98
Nonfermentable sugar, "	—	—	—	—	33
Nonprotein nitrogen, "	—	—	—	54	38
Haemoglobin, %	—	—	17.1	17.3	17.2

Table II. The xylan feeding test.

Date (1930~1932).	1930	1930	1931	1932	1930	1931	1931
	9, 20	10, 1	3, 21	4, 18	10, 3	1, 29	1, 30
Sex of albino rat.	m.	f.	f.	f.	f.	f.	f.
Hours fasted.	23.5	20.0	24.0	22.0	9.0	8.0	8.0
Hours, fed to killed.	2.0	3.5	2.0	2.0	16.0	18.0	16.0
Hours, fasting begun to killed.	25.5	23.5	26.0	24.0	25.0	26.0	24.0
Body weight, g.	152	115	180	145	94	134	145
Xylan eaten, g.	2.0	2.4	2.0	1.9	3.0	2.5	2.4
Liver weight, g.	6.2	5.0	7.1	7.0	3.7	7.0	7.2
Liver glycogen, mg. in 100 g.	15	46	57	61	64	128	115
Muscle weight (calculated), g.	71.4	54.1	84.6	68.2	44.2	63.0	68.2
Muscle glycogen, mg. in 100 g.	16	173	165	171	158	256	148
Total glycogen in 100 g. body.	8.1	83.3	79.8	83.4	76.8	127.0	75.2
Do. average.	8.1		82.2			93.0	
Do. increase compared with fast.	−4.1		2.1			12.9	
Do. increase per 1 g. xylan.	−3.1		1.4			5.8	
Total blood sugar mg. in 100 c.c.	—	100	98	90	86	104	95
Nonfermentable blood sugar. "	—	—	34	—	—	36	32
Nonprotein nitrogen. "	—	—	40	38	—	49	38
Haemoglobin. %	—	—	17.9	16.9	17.9	16.9	17.4
Xylan recovered from dig. tract.	—	—	1.83	1.50	—	—	1.45

Table III. The starch feeding test.

Date. (1930~1932)	'30. Sep. 20	'32. Mar. 29	Apr. 18.	'30. Oct. 31	'31. Jan. 31
Sex of albino rat.	male	female	female	female	female
Hours fasted.	24	24	22	9	8
Hours, fed to killed.	2	2	2.5	16	16
Hours, fasting begun to killed.	26	26	24.5	25	24
Body weight, g.	184	175	140	111	143
Starch eaten, g.	4	4	4	4	2
Liver weight, g.	7.8	7.2	7.2	4.7	7.1
Liver glycogen, mg. in 100 g.	1060	1394	1470	1284	214
Muscle weight (calculated), g.	86.5	82.3	65.8	52.2	67.2
Muscle glocogen, mg. in 100 g.	452	391	264	247	94
Total glycogen in 100 g. body.	257.4	241.3	199.3	170.5	54.8
Do. average.	257.4	220.3		112.7	
Do. increase compared with fast.	245.2	140.2		32.6	
Do. increase per 1 g. starch.	113.0	54.6		13.0	
Total blood sugar, mg. in 100 c.c.	—	109	112	115	103
Nonfermentable blood sugar. "	—	32	—	—	31

Nonprotein nitrogen, mg. in 100c.c.	—	40	39	45	39
Haemoglobin, %	17.4	17.1	16.8	17.2	16.9
Starch recovered from dig. tract.	—	2.07	1.95	—	0.3

The increase in the amount of glycogen by xylan feeding is found to be very small when compared with that in the case of starch feeding. The amount of absorbed xylan too is much smaller than that of absorbed starch. This, by the way, is a fact never noticed in the case of rabbits as was communicated in the first and second report[1)(2)]. Therefore, sueh a comparison should be based on the amount of substances absorbed. The digestibility of xylan two hours after the feeding was found to be 14.6%, and that of starch 49.8%. The digestibilities 16 hours after the feeding were not estimated then. Ordinary digestibility coefficients were, therefore, estimated afterwards, detail accounts of which will be reported in the next paper, but the results are briefly as follows: xylan 33.1%, starch 86.2%. These digestibility coefficients may be applied, for the comparison without any appreciable error, to the case of 16 hours after the feeding. Thus the increase in the amount of glycogen per gram of digested xylan or starch may be shown in Table IV, and figure.

Table IV. The glycogen increase per gram of digested xylan.

	Xylan feeding.			Starch feeding.		
	one ♂	av. three ♀	av. three ♀	one ♂	av. two ♀	av. two ♀
Hours after the feeding.	2.0	2.5	16.6	2.0	2.3	16.0
Glycogen increase mg.	neg.	96	18	227	127	15

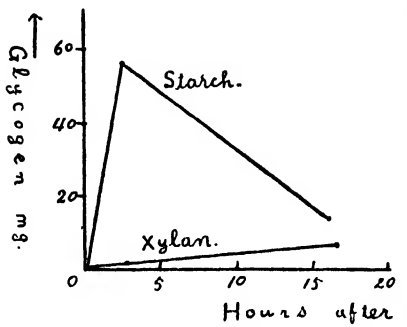

Fig. I. Glycogen increase per gram of eaten material.

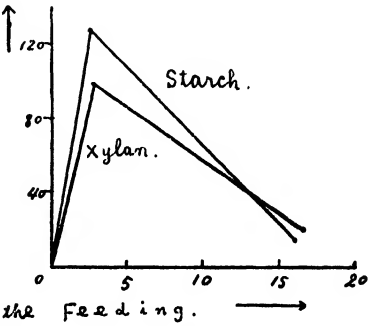

Fig. II. Glycogen increase per gram of digested material.

Conclusion.

1. Glycogen could be accumulated in the body of albino rat by the

feeding of xylan. The accumulation, however, is very small in comparison with the application of starch. This may be attributed mainly to the difficulty in the digestion of xylan by rats. Accordingly, the increase in the amount of glycogen per gram of digested xylan is never so much small in comparison with that caused by the starch.

2. So far as the amounts of nonfermentable sugar, nonprotein nitrogen and haemoglobin are concerned, there was no appreciable difference noticed in the blood of rats, when examined at the end of either 2.5 or 16 hours after the feeding of xylan or starch or none of them. The total blood sugar, however, was found decreased somewhat in the case of xylan.

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On the Nutritive Value of Pentosan. IV.

The Methods of Treatments and the Kinds of Animals as two of the Effecting Factors upon the Digestibility of Pentosan.

By

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(*From the Laboratory of Biochemistry, Morioka Imperial College of Agriculture and Forestry.*)

(Received May 30, 1930.)

The effect of various treatments, such as disintegration with alkaline solutions etc., upon the digestibility of pentosan was studied, using various gramineae straws which were recently applied to the investigation of straw-disintegration by the author[1-5]. The results obtained are shown in Table I. The effects upon the digestibility of crude fiber and nitrogen free extract may be added here for a reference.

Table I.

The effect of various treatments upon the digestibility of pentosan.

Materials and treatments.	Digestibility of pentosan by sheep av. %	Ratio of digestibility (untreated, as 100)		
		Pentosan	Crude fiber	N. free ext.
Rice straw, untreated.	52.6	100	100	100
Do. soaked 4 hours in 0.25% NaOH	53.7	102	101	87
Do. soaked 4 hours in 0.75% NaOH	82.9	158	141	153
Do. soaked 4 hours in 1.5% NaOH	82.1	156	139	156

Do. boiled 3 hours in water.	58.6	111	102	105
Do. boiled 3 hours in 1% CaO	87.9	167	141	139
Do. boiled 1.5 hours in 1% CaO	82.8	157	131	139
Do. soaked 2 days in 1% CaO	79.5	151	130	131
Do. soaked 2 days in wood ash extract.	63.5	121	110	119
Barley straw, untreated.	43.8	100	100	100
Do. boiled 3 hours in 1% CaO	74.8	171	128	125
Do. boiled 1.5 hours in 1% CaO	77.6	177	132	118
Wheat straw, untreated.	56.2	100	100	100
Do. boiled 3 hours in 1% CaO	76.8	137	124	121
Do. soaked 4 hours in 0.9% NaOH	75.8	135	125	129
Coarse hay of "Susuki", untreated	34.8	100	100	100
Do. boiled 2 hours in 1% CaO	72.0	207	156	156
Straw of "Hiye", untreated.	43.9	100	100	100
Do. boiled 2 hours in 1% CaO	80.1	182	154	113

Next, the difference in digestibility of pentosan among rabbits,[6][7] albino rats[8] and guinea pigs was studied. The xylan, isolated from rice straw, was used as an example of pentosans. Experiments were carried out in an usual way, namely, a certain amount of the xylan with basal fodder was fed to each animal. The results obtained are summarised in Table II.

Table II.

The digestibilities of pentosan by rabbits, rats and guinea pig.

	Digestibility of xylan.	Digestibility of starch.
Two rabbits, average %	78.9	83.6
Two albino rats, av. %	33.1	86.2
One guinea pig. %	62.9	—

Conclusion.

1. When the straw of rice, barley, wheat, hiye (*Panicum Crus-Galli*, L. var. frumentaceum, Hook.) or coarse hay of susuki (*Miscanthus sinensis*, Anders.) was either soaked 4 hours in a cold solution of 0.75～1.5% caustic soda, or two days in 1% lime suspension, or boiled 1.5～3 hours in 1% lime, the digestibility of pentosan was found to have become 1.4～2.1 times as that of the untreated material. When the rice straw, however, was either soaked 4 hours in 0.25% caustic soda solution, or two days in wood ash solution, or boiled 3 hours in water, no increase in the digestibility of pentosan was noticed.

2. The increase in digestibility of pentosan was found somewhat greater

than that of crude fiber, or of nitrogen free extract, yet bearling nearly a constant ratio between them.

3. The digestibility of xylan, isolated from rice straw, varied remarkably as the kind of animals used. The rabbit digested 78.9% of xylan, the albino rat 33.1% of it, and the guinea pig 62.9% of it. In the case of potato starch, however, the rabbit digested 83.6%, and the albino rat 86.2% of it.

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(8) Do. : Do. Vol. 8, 90 (1932).

MISSPRINT CORRECTED.

1. First report of this investigation (this journal. Vol. 7, p. 35, 1931.).
   14th line; 3029 calories, should be corrected to read as, 2552 calories.
2. Second report (this journal. Vol. 7, p. 41, 1931).
   8~9th line; by 0.1%, 8~10 hours, should be corrected to read as, by 0.01%, 8~18 hours.

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On the Alcoholic Fermentation of the Amino-acids. Part I.

By

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(Received June 7, 1932.)

It has been shown by F. Ehrlich in a series of famous researches that the alcohols contained in fusel oil come from the amino-acids which are formed by the hydrolysis of the proteins. But little is known about the fermentation products derived from some of the simpler amino-acids, especially of the normal type. Also it has not yet been clear about the origin of normal propyl alcohol though it is thought to be one of the usual constituents of fusel oil.

The author found with vanilline-H_2SO_4 reagent[1] that there was some

production of fusel oil in the fermented, modified Hayduck solution in which several amino-acids were used in place of asparagine; the quantity of produced fusel oil was ca. 0.03~0.1% when glycocoll, *d*-alanine, *d*-*l*-alanine, *d*-*l*-*α*-amino-*n*-butyric acid, *l*-leucine, *d*-*l*-leucine, *d*-*l*-valine and *d*-*l*-*n*-valine were used, while trace in cases of glutamic acid, asparagine, ammonium-phosphate, ammonium-sulphate, yeast-juice and sugar alone.

Vigorous fermentations were observed in cases of alanine, leucine, Am.-phosphate, Am.-sulphate, glutamic acid and asparagine, while glycocoll and valine were proved to be unadequate nitrogen nutriments for yeast, the last substance being the most inferior. The less the yeast inoculated is, the more fusel oil is obtained. Thus it is supposed that if the yeast is too much inoculated, the sugar may be consumed so quickly that no complete decomposition of amino-acid is expected.

Experimental.

1. Fusel oil produced by saké-yeast in the modified Hayduck solutions in which several amino-acids etc. were used in place of asparagine. Fusel oil was estimated with vanilline-H_2SO_4 reagent colorimetrically.

N-nutriment		Age of culture	Yeast	Products		
				Alcohol	Fusel oil	Total acid
Glycine	0.284%	8 days	1 platinum ear	3.85%	0.07%	0.2006
d-alanine	0.337%	6	〃	4.75	0.07	0.1770
Asparagine	0.25%	6	〃	4.85	0.02	0.1652
Glutamic acid-Na	0.64%	6	〃	3.65	0.02	0.2596
Am-phosphate	0.25%	6	〃	2.65	0	0.2360
Am-sulphate	0.5%	6	〃	4.85	0	0.3068
Sugar alone		7	bulk of a pea	1.975	0.003	
r-alanine	0.4%	6	1 platinum ear	5.4	0.10	
r-aminobutyric acid	0.4%	7	3 platinum ears	5.36	0.12	
r-valine	0 35%	18	bulk of a pea	4.5	0.035	
r-*n*-valine	0.34%	10	〃	5.5	0.020	
l-leucine	0.34%	6	1 platinum ear	5.0	0.08	
r-leucine	0.34%	6	〃	5.0	0.07	
Yeast water*		6	〃	5.0	0	
〃 with cells		6	〃	4.6	0	

* 5 g. of the dried saké-yeast is boiled with 100 c.c. of water for 1 h. and then divided into two parts.

2. The relation between yeast quantity and fusel oil formation.

N-nutriment		Age of culturs	Yeast	Products	
				Alcohol	Fusel oil
r-alanine	0.4%	7	1 platinum ear	4.3%	0.050%
〃	〃	4	3 peas of yeast mud	4.3	0.007
l-leucine	0.5%	7	1 platinum ear	4.3	0.075
〃	〃	4	3 peas of yeast mud	4.4	0.030

(1) M. Yamada: Bull. Agricult. Chem. Soc. Japan, **4**, 33~4 (1928); Chem. Zent., II, 2603 (1928).

On the Alcoholic Fermentation of the Amino-acids. Part II.

Alanine.

By

Masakazu YAMADA.

(*Imp. Brew. Experimental Station, Takinogawa machi near Tokyo.*)

(Received June 7, 1932.)

F. Ehrlich obtained *l*-alanine from *r*-alanine when the latter was fermented with the sugar by yeast but did not descrived any volatile products[1]. In that case O. E. Ashdown and J. T. Hewitt perceived that there was some increase of acetaldehyde formation[2] which was now proved to be an ordinary phenomena——that is to say, contrary to their observation the acetaldehyde was not derived from alanine but from alcohol by means of secondary oxidation when yeast came in contact long with the fermented liquid after the alcoholic fermentation[3]. Though the aldehydes such as isobutylaldehyde or isovaleraldehyde have often been found in crude spirit and regarded as the intermediate product from amino-acid to higher alcohol in the so-called alcoholic fermentation of amino-acid, they come actually from the amino-acids by the reaction between amino-acid and sugar in the extractives when heating or distillation is carried out[4]. If alanine be also attacked by yeast according to Ehrlich's view, ethyl alcohol must be the resultant fermentation product. Now the modified Hayduck solution with pure *r*-alanine synthesized from pure ethyl alcohol in place of asparagine was fermented by saké or distillery yeast and the distillate containing some higher alcohols was obtained. Fusel oil fraction which boils over 100° consists mainly of isobutyl-alcohol and the smaller quantity of amyl alcohol. Thus if alanine be the mother substance

of isobutyl alcohol, the substance of four carbon atoms have to come synthetically from the substance of three carbon atoms but the process has not yet been clear. Continued experiments have shown that isobutyl alcohol does never arise from other amino-acids of course containing valine.

Experimental.

1 to 2.5 l. of culture medium in a 2 to 5 liter flask was sterilised as usual by being boiled for 1 h. every three days and then yeast was added. After about 10 days the fermentation was over and the whole sugar was decomposed. Yeast was filtered and the clear filtrate was first distilled in the pot-still of about 15 liters. The distillate then redistilled through the fractional columb with 5 bulbs several times until the last distillate showed no fusel oil reaction with vanilline-H_2SO_4 reagent. The residues of each distillation except the first were collected and redistilled. Yellow oily layer and the ether extract of water layer were mixed, which was then fractionated in a small flask of about 30 c.c. The first distilling residue containing nonvolatile substances was evaporated on the water bath and filtered. Then from the greenish clear filtrate copper was removed with H_2S phosphate with ammonia and the filtrate was evaporated into syrup to which 90% of alcohol was added and was allowed to stand in a cold place for a day. Crystals of amino-acid was disolved in a hot water, decolorized and recrystallized. The result of the fractionation of oil is as follows.

Remarks :-

I, III, V Modified Hayduck solution with alanine in place of asparagine.
II Sugar alone (8%) without nitrogenous substances and mineral matters.
IV Sugar and alanine without mineral matters.
V One platinum ear of yeast is propagated in 100 c.c. of medium which is poured into the 1 liter of medium. The latter is divided among ca. 30 liters.

	I	II	III	IV	V
Yeast inoculated	Sacch. saké No. 1 10 g. as dry yeast	Sacch. saké No. 1 63 g.	Sacch. saké No. 1 27.6 g.	Beer yeast 5575 g. (wet)	Distillery yeast from 1 pt. ear
Alanine	*d*; 0.4%	——	*r*; 0.34%	*r*; 0.4%	*r*; 0.34%
Total medium	30 L.	20 L.	37.56 L.	50 L.	32.2 L.
Period of ferment.	12 days	7	10~12	6	10~12
Temp.	26~8°	27.9°	24~9°	14°	26~8°
Alcohol produced	——	4.0%	4.7%	4.85%	4.5%
Total acid	0.1298%	0.0944%	0.0912	0.0413	0.1062
Aldehyde	0.00241%	——	0.00164	——	0.00453
Fusel oil	0.05%	0.005%	0.07	0.007	0.1
Alcohol (yield)	1410 c.c. (94%)	695 c.c. (94.2%)	1735	3720 (93.5%)	1510 (95%)

Alcohol (yield) (fusel oil)	41 c.c. (3.57 g.)	47 (91.6%) (0.14 g.)	16.5 (1.65 g.)	44 (0.7 g.)	67 (5.9 g.)
Oil	5.35 g.	0.6	6.1	1.8	9.8
Fract. distill. of oil					
100~106°	0.65 g.	0	0.5	0	1.4
105~113°	2.1 (I B)	0	2.3 (III B)	0	4.6 (V B)
113~122°	0.3	0	0.3	0.15	1.8
122~128°	0.6	0.2 (II A)	0.8 (III A)	0.3	0.8 (V A)
128~130°	1.1 (I A)	0.3	0.9	1.0	1.0
Residue	0.25	—	0.5	0.3	0.2
Yield of yeast (dry)		54 g.	110	1650	55
Alanine recovered	30 g.	—	45.6	149	45.5
Rotation (in 20% HCl)			$[\alpha]_D^{22°} = 6.00°$		$[\alpha]_D^{21°} = 6.43°$

Identification of the fractions.

The following derivatives of alcohol were prepared.

Phenylcarbamate :-

Fraction	M. P.	Analysis				
		Subst. g.	N (c.c.)	T.	P. (m.m.)	N (%) found
I B	80°	0.0983	6.2	15°	768	7.46
III B	83°	0.0798	4.9	13	765.5	7.33
V B	83°	0.0854	5.4	18	758	7.31
Isobutyl alcohol	80°					7.25 ($C_{11}H_{15}O_2N$)

3 • 5 • dinitrobenzoate :-

Fraction	M. P.	Analysis				
		Subst. g.	N (c.c.)	T.	P. (m.m.)	N (%) found
I B	84°	0.0536	4.75	16°	764	10.41
I A	57°					
II A	52°					
III B	86°	0.0511	4.55	15	761	10.47
III A	62°	0.0582	4.90	15	762.5	9.92
V B	85°	0.0753	6.85	19	761.2	10.51
V A	61°	0.0525	4.55	22	756	9.79
Isobutyl alcohol	87°					10.45 ($C_{11}H_{12}O_6N_2$)
Isoamyl alcohol	62°					9.93 ($C_{12}H_{14}O_6N_2$)

(1) F. Ehrlich: Biochem. Zs., 1, 8 (1906).
(2) O. E. Ashdown and J. T. Hewitt: J. Chem. Soc, London, 97, 1636~48 (1910).

(3) M. Yamada: Bull. Agricult. Chem. Soc. Japan, 3, 76~80 (1927); Chem. Zent., II, 2479 (1928).
A. Trillat et Sauton: Compt. rend., 146, 996~9 (1908).
(4) M. Yamada: Bull. Agricult. Chem. Soc. Japan, 4, 18~21 (1928); Chem. Zent., II, 585 (1929); Bull. Agricult. Chem. Soc. Japan, 4, 89~91 (1928); also see
S. Akabori: Proc. Imp. Acad. Tokyo, 3, 672~4 (1927); Chem. Zent., I, 1757 (1928).

On the Alcoholic Fermentation of the Amino-acid. Part III.

α-amino-*n*-butyric acid.

By

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(Received June 7, 1932.)

Abderhalden, Chang and Wurm obtained *l*-isomeride as the decomposition product by yeast from *r*-amino-*n*-butyric acid and supposed that *d*-component was the naturally occurring isomeride. But then they did not pay attention to the volatile products. According to Ehrlich's view *n*-propyl-alcohol ought to be produced from the amino acid.

The author's experiment showed that the fusel oil fraction produced by saké yeast in the modified Hayduck solution with this amino acid in place of asparagine consisted mainly of an amyl alcohol which boiled at about 123~8°. From the rotatory power of the alcohol and the properties of two derivatives, this was identified as active amyl alcohol.

Experimental.

d-l-α-amino-*n*-butyric acid was prepared from *n*-propyl alcohol merck.

Medium: Cane sugar 100 g., amino acid 3 g., Hayduck mineral solution 20 c.c., water 980 c.c.

1 to 2.5 l. of culture medium in a 2 to 5 l. flask was sterilized in ordinary way and then saké yeast was added—23 g. as dry yeast for total 13.2 l. of the medium. After 11 days at 25~9° the fermentation was over and the fermented liquid contained no sugar. The distillation and the treatment of the residue are the same as the case of alanine (see part II).

	Alcohol	Total acid	Fusel oil
Fermentation products	4.4%	0.1416%	0.07%
Yield of alcohol	632 c.c. (93%)	24 (90%) (fusel oil 0.768 g.)	oil (B. P. over 100°) 7.3 g.

Fractionation of the oil.

	B. P.	Yield (g.)		B. P.	Yield (g.)
I	100~107°	0.2	V	126~127.5°	1.7
II	107~116°	0.3	VI	127.5~128.5	2.0
III	116~123°	0.1	VII	residue	0.5
IV	123~126°	1.7			

Identification of the fractions.

The following derivatives of alcohol were prepared.

Phenyl carbamate :-

Fract.	M. P.	Analysis				
		Subst. (g.)	N (c.c.)	T.	P. (m.m.)	N (%) found
IV	32.5°	0.0715	4.2	15°	765	6.93
Act. amyl alcohol	30°					6.76 ($C_{12}H_{17}O_2N$)

3-5-dinitrobenzoate :-

Fract.	M. P.	Analysis				
		Subst. (g.)	N (c.c.)	T.	P. (m.m.)	N (%) found
IV	80°	0.0564	4.7	15°	768.3	9.89
V	81.5	0.0666	5.7	15	760.5	10.05
Amyl alcohol						9.93 ($C_{12}H_{14}O_6N_2$)

Rotation of alcohol (mixture of IV, V, VI).

$[\alpha]_D^{23°} = -5.55°$ $r = -3.62°$ $l = 2.2$ dm. $c = 29.64\%$

$[\alpha]_D^{20°} = -5.90°$ (methyl ethyl carbin carbinol).

Amino acid recovered from the distilling residue was 7.6 g. and its rotation in 20% HCl solution was following.

$[\alpha]_D^{22°} = -1.14°$ $r = -0.16°$ $l = 2.2$ dm. $c = 6.37\%$

Chemical Studies of Agar-Agar.

III. On the Isolation of free λ acid from Hydrato-Kanten-λ by means of Electric Dialysis.

By

E. Takahashi and K. Shirahama.

(Received July 4th, 1932)

λ acid (Kanten-acid), a principal constituent of agar-agar and its constitution is denoted by $R{\cdot}O{\cdot}SO_2{\cdot}OH$, was isolated from the hydrato-kanten-λ* (obtained by hot water-hydrolysis of agar-agar) by means of electric dialysis using Pauli's apparatus.

Two methods were adopted for the isolation of this acid.

(1) After treating the dialyzed solution with small quantity of ammonium chloride solution, pourd into 99% alcohol (4～5 volumes of original solution) by which λ acid was precipitated as a ammonium salt.

(2) After adding to the dialyzed solution 4 volumes of 99% alcohol, the same volume of ether, and further 2.5 times as much benzol as total volume. The mixture was condensed to a small volume at 40～50°C under diminished pressure.

Next, 3～4 volumes of ether, was added to the solution by which free λ acid was settled as a white voluminous precipitate.

General natures of various preparations are summarised as Table I : —

Summary.

(1) Aqueous solution of λ acid gives colloid. This acid is stable in the combined form with alkalis or metals. But in the free state, it is unstable and easily decomposable.

(2) The rotately power of this acid shows −31° and its composition as far as made clear is as follows : —

Galactose	39.47%	Pentose	8.17%
SO_4	ca. 8.00%		

Besides above, it contains a substance giving ketose reaction and a hexose any other than galactose. The presence of uronic acid and methylpentose is excluded.

* Previous report. This J. 8 659, (1932).

Table I.

No.	Preparation						Ash %	CO_2 %	$[\alpha]_D$	SO_4 %	Titration acidity for 100 g. Sample	Others
	Concentration of dialysed solution		Strength of Electric Current			Treatment						
	λ g.	Water c.c.	Volt.	m.Amp.	Hour.							
1							6.27	0.76	—	6.26	acidic	Galactose=40.65% Pentose=3.40%
2	10	200	110	2.5	48	NH_4Cl+alcohol	5.85	1.16	—	—	—	Ca=2.65% SO_4=3.02%
3	5	200	110	10	24	NH_4Cl+alcohol	3.68	0.80	—	—	—	
4	15	200	110	50~100	30	NH_4Cl+alcohol	—	—	—	8.36	—	Galactose=39.47% Pentose=8.17%
5	10	200	110	50~100	30	NH_4Cl+alcohol	0.80	0.56	−29.4°	8.72	NaOH=1.56g. (SO_4=3.18 g.)	Ammonia N=0.81% (SO_4=5.55%)
6	10	200	110	50~100	30	After neutralised with NH_4OH NH_4Cl+alcohol	0.77	—	—	7.66	NaOH=0.36g. (SO_4=0.86 g.)	Ammonia N=0.90% (SO_4=6.17%)
7	10	200	110	50~100	30	After neutralised with $Ba(OH)_2$ alcohol	—	—	—	8.66	—	SO_4 to be combined with Ba=0.138%
8	10	200	110	50~100	35	NH_4Cl+alcohol	Trace	—	−31.0°	6.90	NaOH=1.05g. (SO_4=2.52 g.)	Ammonia N=1.27% (SO_4=4.36%)
9	10	200	110	50~100	30	Isolation by mixed solvent	0.59	—	−30.8°	6.62	NaOH=3.13g. (SO_4=7.52 g.)	

Qualitative reaction: —— Reducing power negative; ketose, hexose and pentose positive; methylpentose and uronic acid negative.

On the Formation of Lysolecithin from Egg-yolk Lecithin by Pancreas Extract.

By

Ziro Nikuni.

(Received July, 11th., 1932.)

Since the discovery of lysolecithin by Delezenne and Fourneau (Bull. Soc. Chim. iv. **15**, 421, 1914) many works have been done on this subject and it is now generally recognized that the formation of this substance from lecithin by the action of snake venom is due to an enzyme "Lecithinase" in it.

On the other hand, the existence of an enzyme which splits lecithin into fatty acids, glycerophosphoric acid and cholin, was found by Bókay (H. 1, 157, 1877) in cow pancreas. This enzyme seems to be widely distributed in blood, liver, brain und other organs, as well as in egg-yolk, castor bean and Takadiastase etc. So the question naturally arises whether it is the same with the lecithinase of snake venom, or whether lysolecithin is also formed from lecithin during the digestion with this enzyme.

Contardi and Latzer (Bioch. Z. **197**, 222, 1928) obtained a haemolytic substance from the decomposition products of lecithin by Ricinus lipase. Wohlgemuth (Bioch. Z. **39**, 302, 1912) found a haemolytic substance in human pancreatic juice, and Belfanti (Bioch. Z. **154**, 148, 1924: Z. f. Imm. **44**, 347, 1925; **56**, 449, 1928) isolated lysolecithin from pancreas and salivary glands of horse and cow, and supposed that the latter might be formed by the enzyme contained in these organs, but no decisive proof has been given on this subject.

According to the sugestion of Prof. U. Suzuki, the present author tried to digest egg-yolk with pancreas extract or with commercial pancreatin, and observed that a powerful haemolytic substance was formed during the digestion, which disappeared again on further standing. After many trials, the author succeeded in isolating this substance in crystalline state and proved it to be identical with lysolecithin, formed by snake venom. It was further observed that a haemolytic substance is also formed by commercial Takadiastase in the same way.

Experimental.

(1) Digestion of egg-yolk lecithin with pancreas extract.

(a) The pancreas extract containing lecithinase was prepared according

to the method of Willstätter (H. **125**, 153, 1923). The finely crushed pig pancreas was, after being treated with acetone and ether respectively to remove water and fatty substances, dried in a vacuum desiccator. The brown powder thus obtained was now macerated with 16 volumes of 80% glycerin for 4 hours at 30°, then filtered, and the brown filtrate containing the enzyme was used for the experiment.

(b) Sometimes, the finely minced pancreas paste was directly used for the same purpose.

(c) Fresh egg-yolk was mixed with a N/15 potassium-phosphate solution and digested with the pancreas extract, prepared as mentioned above, at 36～38°, being added with a little toluol. The mixture was frequently shaken, and from time to time a definite amount was taken out from it and added with 95% alcohol. The precipitate formed thereby was filtered off, and the filtrate was evaporated nearly to dryness and treated with a small amount of absolute alcohol, filtered, and to the filtrate an alcoholic solution of cadmium chloride was added, whereby the lysolecithin together with lecithin were precipitated as the double salts. These were collected, dried, and again dissolved in 0.9% NaCl solution and tested for haemolytic power according to the Hirao's method (J. Agr. Chem. Soc. Japan, **6**, 738, 1930), using 2% suspension of red blood corpuscles. The experiment was repeated several times with nearly the same result. For example, one experiment is given below.

Experiment: as the source of lecithinase, either 5 g. of pancreas paste, or 12 g. of the glycerin extract obtained from 5 g. pancreas paste, were used. 5 fresh egg-yolks (85 g.) were well mixed with N/15 potassium-phosphate solution, and filled up to 300 c.c. Each 100 c.c. of this mixture was now treated as follows.

1. Control: 100 c.c. yolk solution and 3 c.c. toluol.
2. Pancreas paste: 100 c.c. yolk solution and 3 c.c. toluol and 5 g. paste.
3. Glycerin extract: 100 c.c. yolk solution and 3 c.c. toluol and 12 g. extract.

The digestion was carried out at 36～38°. Each 5 c.c. was taken out from time to time, and tested for haemolytic power as descrived above.

The results are given in the following table.

Table I.

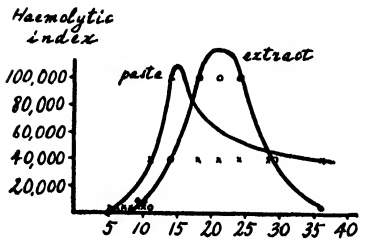
	1	2	3	4	5	6	7	8	9	10
Hours:	15min.	3 hrs.	20 hrs.	2 days	3 days	4 days	5 days	6 days	7 days	8 days
Control:	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace
Paste:	trace	400	trace	400	400	400	4,000	4,000	4,000	4,000
Extract:	trace	trace	trace	trace	trace	400	400	400	400	400

	11	12	13	14	15	16	17	18	19
Hours:	9 days	10 days	11 days	14 days	17 days	21 days	24 days	29 days	36 days
Control:	trace	trace	trace	trace	400	400	400	trace	
Paste:	4,000	4,000	40,000	100,000	40,000	40,000	40,000	40,000	40,000
Extract:	4,000	4,000	4,000	40,000	100,000	100,000	100,000	40,000	4,000

The numbers of the table indicate the haemolytic index (Kofler: Die Saponine, 148, 1927). For instance, the number 40,000 shows that the 1/40,000 solution of the sample to be tested causes the complete haemlysis.

The results are shown graphically in Fig 1.

Fig. 1.



The results show that, during the digestion of egg-yolk with pancreas extract, a substance of strong haemolytic power is formed. The highest activity being attained after 10～20 days. After that time, it decreases rapidly and finally disappears.

(2) Digestion of egg-yolk lecithin with commercial pancreatin.

(a) The above experiment was repeated with commercial pancreatin, prepared by Konishi & Co. according to Japanese Pharmacopoea in the following way : fresh pig or cow pancreas is finely crushed and macerated with two parts of water, saturated with chloroform. After standing for 12 hours it is filtered from insoluble residue, the oparescent filtrate is once more filtered and evaporated to dryness at 45°. In this way a yellowish white powder having a characteristic smell and flesh-like taste is obtained. It is completly soluble in water, but insoluble in alcohol.

(b) 5 yolks were mixed with N/15 potassium-phosphate solution and filled up to 300 c.c. For the experiment, 100 c.c. of this solution were digested with 1/2 g. pancreatin, being added with 3 c.c. toluol. At the same time, another 100 c.c. of the solution were treated with viper venom, obtained from one viper (Agkistrodon blomhoffii) under the same condition. The digestion was carried out at 36～38° and the haemolytic power was determined with the following results.

Table 2.

	1	2	3	4	5	6	7
Hours:	5 min.	2 hrs.	19 hrs.	2 days.	3 days.	4 days.	6 days.
Viper venom:	40,000	40,000	40,000	40,000	100,000	100,000	100,000
Pancreatin:	0	40,000	40,000	40,000	100,000	200,000	200,000

	8	9	10	11	12	13	14
Hours:	8 days.	10 days.	13 days.	16 days.	21 days.	28 days.	39 days.
Viper venom	100,000	60,000	50,000	40,000	40,000	4,000	4,000
Pancreatin	200,000	100,000	100,000	100,000	80,000	100,000	100,000

This results show that the haemolytic substance of high activity was formed during the digestion. The activity reaches the maximum in 5～6 days and afterwards decreases rapidly. It is also noteworthy that the curves of pancreatin and viper venom in figure 2 show remarkable similality.

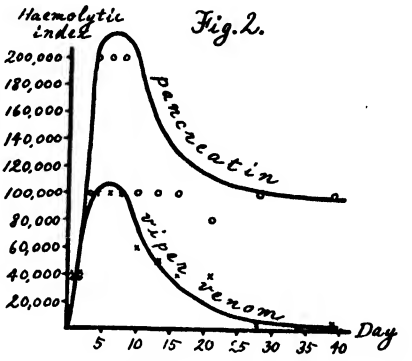


Fig. 2.

(3) Isolation of lysolecithin from the digestion liquid of egg-yolk with pancreatin.

The method adopted in this experiment was essentially the same with that of Mr. Iwata (J. Agr. Chem. Soc. Japan, **6**, 759, 1930: **7**, 557, 1931; Bioch. Z., **224**, 430, 1930).

30 fresh yolks (515 g.) were mixed with 500 c.c. of N/15 potassium-phosphate solution and treated with 5 g. commercial pancreatin. The mixture was kept at 36～38° for 2½ hours with frequent shaking, no antiseptic being used in this case. It was then poured into an equal volume of warm 95% alcohol, filtered, the residue was washed twice with each 400 c.c. 85% alcohol. Both filtrate and washings were united and evaporated in vacuum until about 80 gramms thick brown syrup was obtained. This syrup was again dissolved in 200 c.c. absolute alcohol and filtered, the insoluble residue was washed three times with each 20 c.c. absolute alcohol. The combined filtrate thus obtained was now added with 15 g. cadmium chloride, dissolved in 250 c.c. hot absolute alcohol, whereby a white voluminous precipitate was formed. After addition of 300 c.c. acetone, the cadmium precipitate was filtered, washed five times with a mixture of alcohol and acetone, and dried in a vacuum desiccator. A white powder was thus obtained. The yield was 31 g. and the haemolytic index about 60,000.

The cadmium double salts, obtained as above was now suspended in a mixture of 100 c.c. chloroform and 50 c.c. of absolute alcohol, and decomposed by adding 80 c.c. of 4% alcoholic ammonia. The precipitated cadmium hydroxide was centrifuged off and the clear filtrate was evaporated to dryness, treated with 50 c.c. absolute alcohol, filtered from insoluble residue and the clear brown filtrate was poured into 1½ litre ether, whereby the crude

lysolecithin was precipitated: yield 5½ g.

This crude product was recrystallized from absolute alcohol, alcohol and chloroform, pyridin, chloroform, alcohol and acetone and finally from alcohol and chloroform. In this way the lysolecithin was obtained as colourless nice prisms. Yield ½ g.

The lysolecithin, purified as above, is slightly hygroscopic. Heated in a sealed capillary, it begins to soften at 95° and decomposes to a brown liquid at 262~263°. The haemolytic index is 320,000, Mixed with the lysolecithin prepared by Mr. Iwata, using cobra venom, no depression of decomposition point was observed. The analysis gave the following results.

Sample	Method	C%	H%	P%	N%	Amino N / Total N
(1) 4.188 mg.	Liebig's	58.24	10.26			
(2) 4.119 mg.	〃	58.16	10.23			
(3) 5.144 mg.	Lieb's			5.95		
(4) 4.680 mg.	Dumas'				3.00	
(5) 5.941 mg.	〃				2.97	
(6) 10.308 mg.	van Slyke's					3/100
mean		58.20	10.24	5.95	2.98	3/100
theory ($C_{24}H_{50}PNO_7$)		58.12	10.17	6.26	2.82	0/100

Summary.

When egg-yolk lecithin was digested with pancreas extract or commercial pancreatin, a powerful haemolytic substance was formed as the intermediate product.

This substance was isolated in pure crystalline state, and was proved to be lysolecithin. It was identical in every respect with lysolecithin formed from lecithin by the action of snake venom.

The author expresses his sincere thanks to Prof. U. Suzuki for his kind guidance throughout this work.

(June 20 th, 1932, Agr. Chem. Lab. Tokyo Imperial University, Komaba)

The Quantitative Studies of some Chemical Constituents of the Mulberry Leaves. Part I.

The Study of the Cystine- and Cysteine-Compounds in the Mulberry Leaves.

By

YUKITARO KISHI.

(Received August 9 th, 1932).

Résumé.

(1) For the purpose of studing mulberry culture, I made a quantitative study of the cystine- and cysteine-compounds in the mulberry leaves, using Okuda's S. S. A. method[1].

(2) Mulberry trees are so vigorous in growth that, even if the stems are cut off close at the stub twice or thrice in succession in a year, new buds form more readily and the leaves and stems thus produced grow more vigorously and luxuriantly than in the case of many other trees similarly treated; therefore, the quantity of the cystine- and cysteine-compounds in the fresh matter of the leaves is small in the buds and very young leaves, but increases in proportion to the growth of the leaves, and reaches the maximum quantity at the part where the action of the synthesis is most vigorous, and then decreases again in the fresh matter of the old leaves. In these compounds in the mulberry leaves, the SH form is found present in greater quantity than the S–S form at each stage of the growth of the leaves.

(3) The quantitative difference of the cystine- and cysteine-compounds contained in the leaves depends on the varieties of the mulberry trees; this is especially so in the young leaves. In the leaves of "Roso", the amount of these compounds tends to be smaller than in the leaves of other varieties; this is especially so in the young leaves used to feed young silkworms.

(4) My experiment has shown that a "hishage" stem (without leaves) of a mulberry tree contains a greater quantity of the cystine- and cysteine-compounds than a healthy stem (without leaves); while, on the contrary, the leaves of the "hishage" stem contains a smaller quantity of these compounds than the leaves of the healthy stem.

The "hishage" is an epithet given by many Japanese sericulturists to a

(1) Journal of the Department of Agriculture, Kyûshû Imperial University, Vol. 2, No. 5, (1929); also Journal of the Agricultural Chemical Society of Japan, 3, 1907, (1927).

physiologically unsound stem. The "hishage" stem is flattened as if crushed, and is fatter in the affected parts than a healthy stem.

Addition: — In studying the amount of the cystine- and cysteine-compounds contained in the mulberry leaves, I have also examined the amount of these compounds contained in a silkworm made to abstain from food. I found that the quantity of the S–S form increases gradually, contrary to that of the SH form.

The Quantitative Studies of some Chemical Constituents of the Mulberry Leaves. Part II.

The Acid–base Balance of the Ash in the Mulberry Leaves.

By

YUKITARO KISHI.

(Received August 9 th, 1932).

Résumé.

(1) I made a study of the acid–base balance of the ash in the mulberry leaves.

(2) The total alkalinity of the ash in the mulberry leaves was determined after incineration, with or without the addition of magnesium nitrate. In either instance, the alkalinity increased in proportion to the growth of the mulberry leaves, as the contents of the ash in the leaves increase in proportion to the growth.

(3) The total alkalinity of the ash in the mulberry leaves treated with magnesium nitrate is generally a little less than that obtained by the ordinary method.

(4) The alkalinity of the ash which is soluble in water decreased in proportion to the growth of the mulberry leaves, as against the total alkalinity in the two instances mentioned above, which increased in proportion to the growth. Consequently, the alkaline ash constituents which is insoluble in water showed an increase in proportion to the growth of the leaves.

Isolation of "Oryzanin" (Antineuritic Vitamin) from Rice-polishings. (Second Report.).

By

Sator Ohdake.

(Agricultural Chemical Laboratory, Tokyo Imperial University, Faculty of Agriculture, Komaba, Tokyo.)

(Received August 10 th, 1932).

In the previous report[1], the author stated that the antineuritic substance was isolated in crystalline state as hydrochloride from rice-polishings and the molecular formula $C_6H_8N_2O_2$ was assigned to this substance, the formula being different from that given by Jansen and Donath[2].

During the studies on sulphur compounds of yeast extract, U. Suzuki and the author[3] found that the antineuritic preparation, "crude oryzanin", obtained from rice-polishings as well as from yeast-extract gave the reaction of sulphur and observed that the antineuritic activity was always accompanied by the sulphur reaction. Reminding this fact, the author detected the presence of sulphur in the active hydrochloride refered to above and the molecular formula given in the first report had to be corrected as $C_{12}H_{16}N_4SO_2$–2HCl.[4]

A. Windaus and his coworkers[5] reported, recently, that they isolated the antineuritic vitamin in the form of picrolonate from yeast extract. They stated that the antineuritic yeast vitamin contains sulphur which is detectable by boiling with a strong alkali solution in the presence of lead acetate and the active substance should be designated by the formula $C_{12}H_{17}N_3SO$ by analysing the picrolonate. Although the analytical results do not agree with each other it is presumed, from chemical properties, that the antineuritic yeast vitamin isolated by Windaus would be identical with oryzanin isolated from rice-polishings by the present author. Van Veen[6] in Java also reported that

(1) The proceeding of the Imperial Academy of Japan, **7** (1931), No. 3, 102~105 ; J. Agr. Chem. Soc. Japan, **7** (1931), 775~808.

(2) B. C. P. Jansen and W. F. Donath: Mededeeling van den Dinst der Volksgezondheid in Ned-Indie. Anno. (1927), Part I, 1~15.

(3) U. Suzuki, S. Ohdake u. T. Mori: Biochem. Z. **154**, (1924), 280; J. of Agr. Chem. Soc. of Japan. **1** (1924), 1~10.

(4) Bul. of Agri. Chem. Soc. Japan, **8**. 1~3 (1932), 11~46.

(5) A. Windaus, R. Tschesche, H. Ruhkopf, F. Laquer. & F. Schultz: Zeit. fur physiol. Chem., **204** 3~4, (1932), 123~128.

(6) A. G. Van Veen: Zeit. fur physiol. Chem., **208** (1932), 125~128.

he found the existence of sulphur in the antineuritic hydrochloride isolated from rice-polishings and corrected his previous formula as $C_{12}H_{20}N_4SO_2$–2HCl which resembles closely with that given by the present author.

In order to confirm the composition of the active substance, the author prepared, further, sufficient quantity of the active hydrochloride in pure state and compared it, to the affirmative effect, with the preparations purified through picrolonate and chloraurate. Besides the hydrochloride ($C_{12}H_{16}N_4SO_2$–2HCl) the author prepared the picrolonate ($C_{12}H_{16}N_4SO_2$–$2C_{10}H_8N_4O_5$), the picrate ($C_{12}H_{16}N_4SO_2$–$2C_6H_3N_3O_7$), the chloraurate ($C_{12}H_{16}N_4SO_2$–$2HAuCl_4$), and the chlorplatinate ($C_{12}H_{16}N_4SO_2$–H_2PtCl_6) and found always that the base has the composition of $C_{12}H_{16}N_4SO_2$.

Experimental.

The preparation of the active hydrochloride: — In the present work, so-called "Oryzanin-extract", an antineuritic concentrate of rice-polishings was fractionated directly by silver nitrate and baryta to simplify the previous process. The silver precipitates, obtained at the P_H. 4.5∼6.8 was decomposed with dilute hydrochloric acid and it was treated successively with phosphotungstic acid, alcohol, and platinum chloride in alcohol and finally the hydro chloride was crystallized out from alcoholic solution by adding acetone as described in the previous paper. The crude crystals of the hydrochloride were purified by repeating the recrystallisation from alcohol and acetone, untill the crystals showed the constant melting point and revealed to be quite uniform under the polarisation microscope. The yield: 1.6 g. from 11.500 kg. of original rice-polishings.

The hydrochloride prepared in this way possessed entirely the same properties with that purified further through picrolonate and chloraurate. The analytical results also agreed with the formula $C_{12}H_{16}N_4SO_2$–2HCl given in the previous work.

The properties of the hydrochloride: — The purified hydrochloride crystallises in colorless long plates (Fig 1), melting with decomposition at 249∼250°C (uncorr.). It is readily soluble in water, sparingly in alcohol, but insoluble in acetone, ether and benzene etc.

The aqueous solution of the hydrochloride gives precipitates with phosphotungstic acid, mercuric chloride, silver nitrate and baryta, picrolonic acid, platinum chloride, gold chloride, iodine potassium iodide and Dragendorff's reagent, but gives no precipitate with picric acid, lead acetate, tannic acid, mercuric sulphate or with flavianic acid. It gives sulphur reaction which is detectable by giving a violet coloration with sodium nitroprusside or by giving black precipitates with lead acetate when it is boiled with alkali or fused

with metallic sodium previously, while the substance itself gives no reaction with these reagents or with barium chloride in the aqueous solution itself.

It gives Pauly's diazo-reaction, though the coloration is quite different comparing with that given by histidine, histamine or thymine. When it is added with the reagent, it gives yellowish coloration for few minutes which turns to red gradually. It gives also a weak greenish coloration by ferriferricyanide reagent or by phosphomolybdic acid and ammonia while the ninhydrin reaction, purin-reactions, i. e. Kossel's, Weidel's and xanthin-reactions, as well as the arginine reaction by α-naphtol and sodium hypochlorate are all negative.

Rotatory power was measured with 30.995 mg. of the hydrochloride in 1.50887 g. (26°C) of water but no rotation has been observed.

Ultraviolet absorptions spectrogram was taken with mol/10.000. aqeous solution of the hydrochloride, the absorption maxima was exhibited at 270 mμ. (the broad band extending 280～260 m μ.) and 239 m μ. (extending 230～244 m μ.)[7] (Fig. 7). Irradiation destroyed the activity of the hydrochloride.

Analysis of the hydrochloride : —

No.	Subst mg.	CO_2 mg.	H_2O mg.	C%	H%	N%	S%	Cl%
(A) M.Pt. 250°C (Lot No. 1～5.)								
(1)	4.230	6.286	2.090	40.53	5.49	—	—	—
(2)	4.383	6.455	2.194	40.17	5.56	—	—	—
(3)	2.859	0.379 c.c. N (16°C 755 mm.)		—	—	15.55	—	—
(4)	2.734	0.365 c.c. N (15°C 757 mm.)		—	—	15.75	—	—
(5)	4.311	3.718 mg. AgCl		—	—	—	—	21.32
(6)	5.374	3.614 mg. $BaSO_4$		—	—	—	9.23	—
(B) M.Pt. 249°C (Lot. No. 6.)								
(7)	5.412	8.225	2.714	41.44	5.57	—	—	—
(8)	5.235	7.949	2.471	41.41	5.25	—	—	—
(9)	5.194	7.837	2.656	41.15	5.68	—	—	—
(10)	4.385	0.604 c.c. N (15°C 757 mm.)			—	16.26	—	—
(11)	4.915	0.676 c.c. N (12°C 752 mm.)			—	16.29	—	—
(12)	4.287	0.578 c.c. N (15.5°C 756 mm.)			—	15.87	—	—
(13)	5.499	4.413 mg AgCl		—	—	—	—	19.84
(14)	5.261	4.236 mg AgCl		—	—	—	—	19.91
(15)	6.031	3.996 mg $BaSO_4$		—	—	—	9.10	—
(16)	5.668	3.758 mg $BaSO_4$		—	—	—	9.11	—

(C) M.Pt. 250°C (Lot No. 7)

(7) The spectrogram was taken by Dr. M. Sumi with Hilger's quartz spectrograph ; Cf. Guha : Biochem. J. **25** (1931), 941 ; A. Windaus & his co-workers : Zeit. Physiol. Chem. **204** (1932), 127.

(17)	5.068	7.602	2.463	40.99	5.40	—	—	—
(18)	5.145	7.765	2.573	41.16	5.56	—	—	—
(19)	4.253	0.598 c.c. N (23°C 752 mm.)		—		16.04	—	—
(20)	4.342	0.617 c.c. N (25°C 752 mm.)		—		16.10	—	—
(21)	4.879	0.674 c.c. N (23°C 753 mm.)		—		15.78	—	—
(22)	4.995	3.363 mg. $BaSO_4$		—	—	—	9.25	—
(23)	5.942	3.958 mg. $BaSO_4$		—	—	—	9.15	—
(24)	6.016	4.930 mg. AgCl		—	—	—	—	20.26
(25)	5.513	4.489 mg. AgCl		—	—	—	—	20.13
(D) M.Pt. 249~250°C "Purified through Picrolonate".								
(26)	4.355	6.491	2.177	40.65	5.55	—	—	—
(27)	4.863	7.224	2.458	40.51	5.62	—	—	—
(28)	4.270	6.418	2.166	40.99	5.64	—	—	—
(29)	4.504	0.637 c.c. N (25°C 749 mm.)		—		15.96	—	—
(30)	4.600	0.647 c.c. N (24°C 751 mm.)		—		15.97	—	—
(31)	4.194	0.578 c.c. N (23°C 753 mm.)		—		15.74	—	—
(32)	5.078	3.469 mg. $BaSO_4$		—	—	—	9.38	—
(33)	5.364	3.643 mg. $BaSO_4$		—	—	—	9.33	—
(34)	4.834	3.907 mg. AgCl		—	—	—	—	19.98
(35)	5.002	4.055 mg. AgCl		—	—	—	—	20.04
(E) M.Pt. 250° "Purified through chloraurate"								
(36)	5.526	8.302	2.768	40.97	5.53	—	—	—
(37)	3.160	0.451 c.c. N (25°C 752 mm.)		—		16.17	—	—
(38)	5.077	3.490 mg. $BaSO_4$		—	—	—	9.44	—
(39)	4.210	3.428 mg. AgCl		—	—	—	—	20.11
Cal. for $C_{12}H_{16}N_4SO_2$-2HCl				40.79	5.10	15.86	9.07	20.11
Cal. for $C_{12}H_{18}N_4SO_2$-2HCl				40.56	5.63	15.78	9.01	20.00

These results agree most closely with $C_{12}H_{16}N_4SO_2$-2HCl.

The activity of oryzanin hydrochloride: — The antineuritic acitivity of the hydrochloride was tested on pigeons and albino rats with the following results: — (1) To pigeons suffering from polyneuritis by exclusive feeding on polished rice, 0.008~0.01 mg. of the hydrochloride were injected daily. The symptoms were improved in a few hours and the pigeons cured from polyneuritis soundly in 1~2 days. (Chart. 1.)

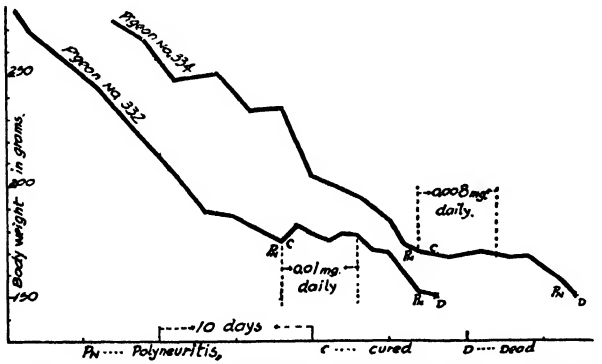


Chart. 1—Pigeons on polished rice & Oryzanin hydrochloride.

(2) Pigeons fed on the artificial diet[8], consisting of 60% purified starch, 20% purified casein, 15%

(8) Chick and Roscoe: Bioch. J. **22.** (1928), 790.

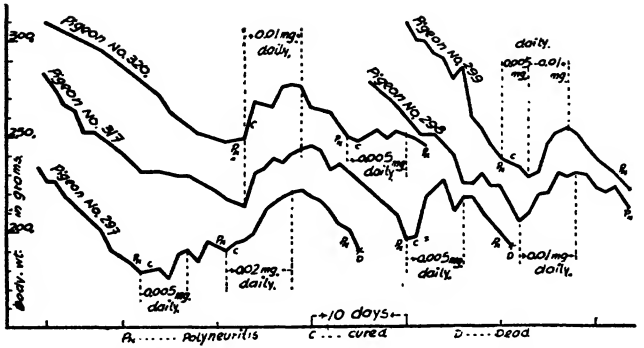


Chart. 2—Pigeons on artificial diet & Oryzanin-hydrochloride.

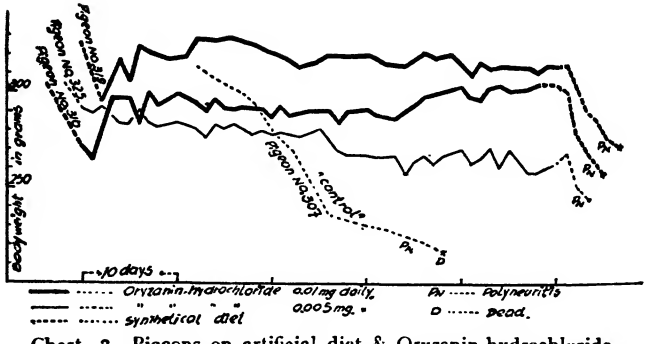


Chart. 3—Pigeons on artificial diet & Oryzanin-hydrochloride.

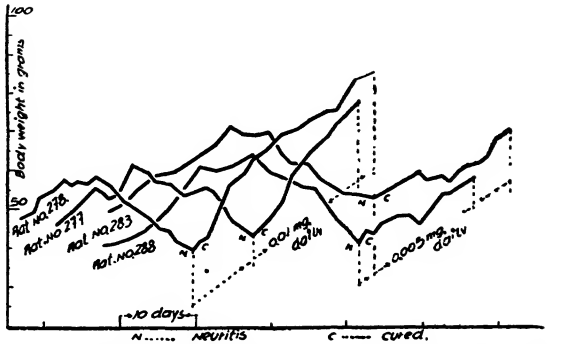


Chart. 4—Rats on artificial diet & Oryzanin-hydrochloride.

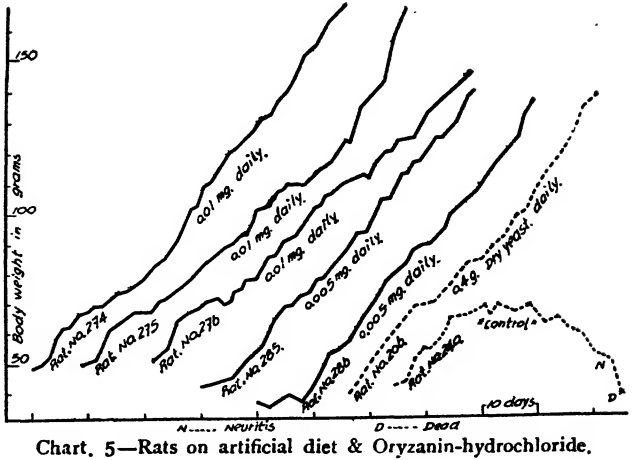


Chart. 5—Rats on artificial diet & Oryzanin-hydrochloride.

peanuts oil and 5% McCollums salt mixture, supplemented with 0.4 g. of autoclaved yeast daily, exhibited the typical symptoms of polyneuritis after 3 weeks usually. By injecting 0.005～0.01 mg. of the hydrochloride daily, the main symptoms were improved in 2～3 hours and cured in 1～2 days. (Chart. 2).

(3) Pigeons fed on the same artificial diet and administered daily with 0.005～0.01 mg. of the hydrochloride in aqueous solution per os, remained in perfect health for 50 days. (Chart. 3).

(4) Young rats, about 40～50 g. in weight, fed on the same artificial diet, supplemented with 3 drops of cod liver oil and 0.4 g. of autoclaved yeast daily, exhibited the symptoms of neuritis in 5 weeks commonly. By supplementing with 0.005－0.01 mg. of the hydrochloride daily, they were cured quickly and their growth curves paralleled almost to the normal. (Chart. 4).

(5) Young rats, fed on the same diet as above, but supplemented daily with 0.005～0.01 mg. of the hydrochloride from the begining of the experiment, grew normally with perfect health for 60 days. (Chart. 5).

(6) The curative day-dose[9] for a pigeon was found to be of the order of 0.0025 mg. as shown in the following table.

Pigeon. No.	Body-weight g.	Days to polyneuritis	Body-Weight suffering from polyneuritis	Dose injected mg.	Days of protection	Day-dose [Dose/Days, protected] mg.	
333	342	22	191	0.012	4	0.0030	
310 (i)	321	24	202	0.01	3	0.0033	
310 (ii)	321	27	199	0.01	3	0.0033	
331	289	22	177	0.01	5	0.0020	
332	285	18	179	0.01	4	0.0025	
334	274	18	185	0.01	5	0.0020	Average 0.0025
311	328	34	181	0.008	3	0.0027	
313 (i)	304	32	172	0.008	3	0.0027	
313 (ii)	304	35	176	0.008	3	0.0027	
324 (i)	342	26	205	0.008	4	0.0020	
324 (ii)	342	30	194	0.008	4	0.0020	
326	317	27	205	0.008	3	0.0027	
330	291	28	184	0.005	3	0.0017	

The picrolonate of oryzanin : —

When the alcoholic solution of picrolonic acid was added to the aqueous solution of the hydrochloride, the light yellow picrolonate separated out which was collected and recrystallised from hot dilute alcohol. It crystallises in

(9) Kinnersly and Peters: Bioch. J. 19 (1925), 820.

light yellow needles (Fig. II) or prisms (Fig. III) and melts sharply at 226°C with decomposition evolving gas. It is readily soluble in hot alcohol, sparingly in water but insoluble in ether, benzene etc. Dried at 100°C in vacuum and analysed.

No.	Subst. mg.	CO_2 mg.	H_2O mg.	C%	H%	N%	S%	Picrolonic acid %
(1)	5.882	10.270	2.266	47.62	4.28	—	—	—
(2)	5.145	8.989	1.950	47.65	4.21	—	—	—
(3)	4.955	0.848 c.c. N (15°C 755 mm.)		—	—	20.15	—	—
(4)	4.919	0.857 c.c. N (16°C 756 mm.)		—	—	20.47	—	—
(5)	4.382	0.764 c.c. N (15°C 752 mm.)		—	—	20.45	—	—
(6)	8.505	2.263 mg. $BaSO_4$		—	—	—	3.64	—
(7)	8.520	2.320 mg. $BaSO_4$		—	—	—	3.74	—
(8)	141.5	92.7 mg. Picrolonic acide.		—	—	—	—	65.51
(9)	161.7	106.7 mg. "	"	—	—	—	—	65.99
Cal. for $C_{12}H_{16}N_4SO_2$-$2C_{10}H_8N_4O_5$				**47.53**	**3.96**	**20.79**	**3.96**	**65.53**
Cal. for $C_{12}H_{18}N_4SO_2$-$2C_{10}H_8N_4O_5$				**47.48**	**4.20**	**20.74**	**3.95**	**65.18**
Cal. for $C_{12}H_{17}N_3SO$-$2C_{10}H_8N_4O_5$ (By Windaus & his coworkers)				**49.29**	**4.24**	**19.77**	**4.11**	**67.73**

The picrate of oryzanin : —

The picrate was prepared from the hydrochloride by adding natrium picrate in dilute alcohol. The light yellow picrate sepatated out, was collected and recrystallised from hot dilute alcohol. It crystallises in light yellow plates (Fig. IV), melting with decomposition at 208°C and it is soluble in alcohol, sparingly in water, but insoluble in ether, benzene etc.
Dried at 100°C in vacuum and analysed : —

No.	Subst. mg.	CO_2 mg.	H_2O mg.	C%	H%	N%	S%
(1)	5.021	7.278	1.375	39.53	3.04	—	—
(2)	5.133	7.441	1.517	39.54	3.28	—	—
(3)	5.197	7.683	1.488	40.03	3.18	—	—
(4)	3.965	0.627 c.c. N (14°C 765 mm.)		—	—	18.93	—
(5)	4.337	0.701 c.c. N (14°C 764 mm.)		—	—	19.32	—
(6)	7.850	2.459 mg. $BaSO_4$		—	—	—	4.17
(7)	8.829	2.708 mg. $BaSO_4$		—	—	—	4.21
Cal. for $C_{12}H_{16}N_4SO_2$-$2C_6H_3N_3O_7$				**39.02**	**2.98**	**18.97**	**4.34**
Cal. for $C_{12}H_{18}N_4SO_2$-$2C_6H_3N_3O_7$				**38.92**	**3.24**	**18.92**	**4.32**

The chloraurate of oryzanin : —

The chloraurate crystallises in light orange yellow long plates (Fig. V) melting at 189°C with decomposition. It is soluble in hot water and in

hot alcohol but sparingly in cold, and insoluble in ether, benzene etc.

The analysis of the chloraurate : —

No.	Subst. mg.	CO_2 mg.	H_2O mg.	Au mg.	C%	H%	N%	S%	Au%	Cl%
(1)	7.517	4.000	1.333	3.146	14.51	1.97	—	—	41.85	—
(2)	7.548	4.084	1.336	3.160	14.76	1.97	—	—	41.86	—
(3)	7.814	4.194	1.326	3.240	14.64	1.89	—	—	41.46	—
(4)	5.780	0.255 c.c. N (15°C 751 mm.)			—	—	5.94	—	—	—
(5)	7.355	0.858 c.c. N (15°C 752 mm.)			—	—	5.71	—	—	—
(6)	9.066	2.300 mg. $BaSO_4$		3.784	—	—	—	3.49	41.74	—
(7)	9.434	2.314 mg. $BaSO_4$		3.937	—	—	—	3.37	41.73	—
(8)	4.507	5.409 mg. AgCl		1.884 mg. Au		—	—	—	41.80	29.67
(9)	4.542	5.395 mg. AgCl		1.897 mg. Au		—	—	—	41.77	29.39
Cal. for $C_{12}H_{16}N_4SO_2$-2HAuCl$_4$					**15.00**	**1.83**	**5.83**	**3.33**	**41.04**	**29.58**
Cal. for $C_{12}H_{18}N_4SO_2$-2HAuCl$_4$					**14.97**	**2.08**	**5.82**	**3.33**	**40.96**	**29.52**

The chlorplatinate of oryzanin : —

Redish orange prisms (Fig. IV) blackening at 245~250°C without melting. It is soluble in hot water but very sparingly in cold, and insoluble in alcohol, ether, benzene etc. Dried at 100°C in vacuum and analysed : —

No.	Subst. mg.	CO_2 mg.	H_2O mg.	Pt mg.	C%	H%	N%	S%	Cl%	Pt%
(1)	7.675	5.107	1.555	1.911	20.87	2.59	—	—	—	28.63
(2)	5.471	0.382 c.c. N (14°C 764 mm.)			—	—	8.35	—	—	—
(3)	6.317	0.449 c.c. N (15°C 751 mm.)			—	—	8.32	—	—	—
(4)	5.233	0.3606 c.c. N (16°C 754 mm.)			—	—	8.08	—	—	—
(5)	8.573	2.946 mg. $BaSO_4$		2.439	—	—	—	4.72	—	28.45
(6)	4.496	5.616 mg. AgCl		1.276	—	—	—	—	31.11	28.38
Cal. for $C_{12}H_{16}N_4SO_2$-H_2PtCl_6					**20.87**	**2.61**	**8.12**	**4.64**	**30.87**	**28.26**
Cal. for $C_{12}H_{18}N_4SO_2$-H_2PtCl_6					**20.81**	**2.89**	**8.09**	**4.62**	**30.78**	**28.18**

Free base of oryzanin : —

A hygroscopic amorphous substance, solubje in water, alcohol, methyl-alcohol, and in acetone, but insoluble in ether, benzene etc.

Summary.

(1) The hydrochloride of oryzanin which was prepared in pure state directly from so-called "Oryzanin-extract" by silver-fractionation, platinum precipitation in alcohol and repeated recrystallisation from alcohol and acetone, possessed entirely the same properties with that purified further through picrolanate and chloraurate. The analytical results agree with the formula

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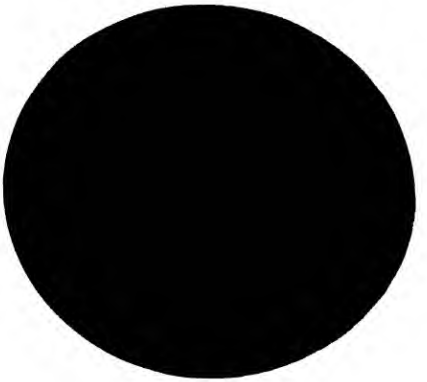
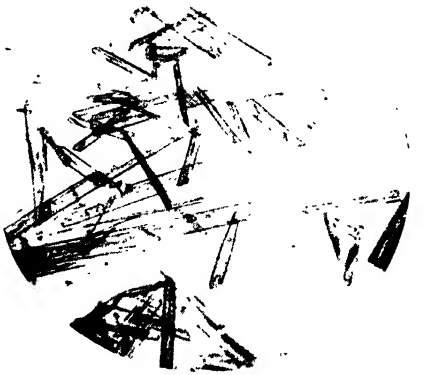


Hydrochloride of Oryzanin.
Leitz. III×2
Fig. I

Picrolonate of Oryzanin, in needles.
Leitz. III×2
Fig II

Picrolonate of Oryzanin, in prisms.
Leitz. III×2
Fig. III

Picrate of Oryzanin.
Leitz. I×2
Fig. IV.



Chloraurate of Oryzanin.
Leitz. I×2
Fig. V.

Chlorplatinate of Oryzanin.
Leitz. III×2
Fig. VI

S. Ohdake: Isolation of "Oryzanin" from Rice-polishings.

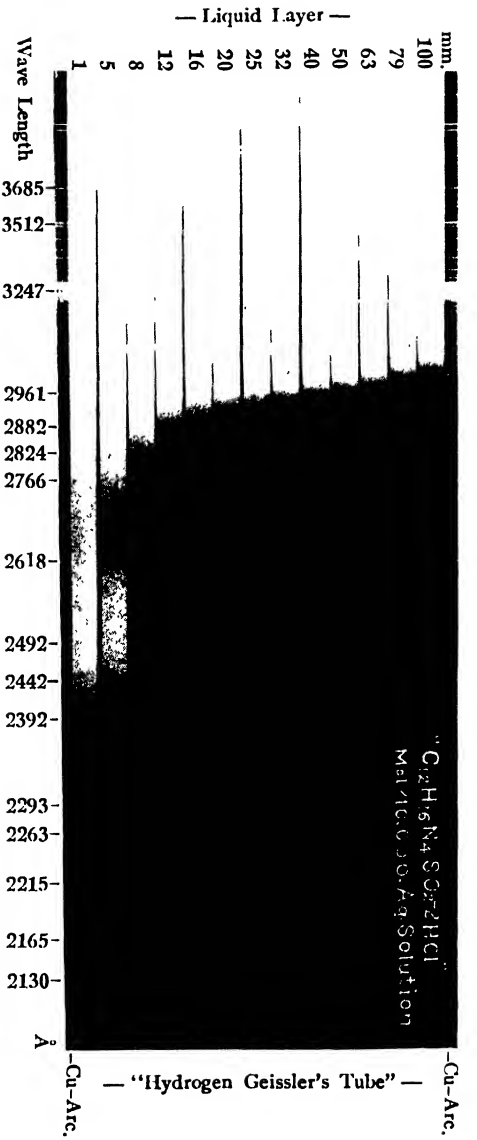


Fig. VII. Ultraviolet Absorptions Spectrogram of Oryzanin Hydrochloride.

$C_{12}H_{16}N_4SO_2$–2HCl which was given by the author in the previous report. The yield: 1.6 g. from 11.500 kg. of rice-polishings.

(2) The hydrochloride cured and protected pigons as well as albino-rats from polyneuritis with the daily dose of 0.005～0.01 mg. The curative day-dose for a pigeon was found to be the order of 0.0025 mg.

(3) Besides the hydrochloride, the author prepared the picrolonate ($C_{12}H_{16}N_4SO_2$–$2C_{10}H_8N_4O_5$ M.Pt. 226°C), the picrate ($C_{12}H_{16}N_4SO_2$–$2C_6H_3N_3O_7$ M.Pt. 208°C), the chloraurate ($C_{12}H_{16}N_4SO_2$–$2HAuCl_4$ M.Pt. 189°C) the chlor-platinate ($C_{12}H_{16}N_4SO_2$–H_2PtCl_6) and finally the free base (amorphous), and studied their properties.

(4) From these results, it was confirmed that the antineuritic substance isolated from rice-polishings by the author, is a new sulphur compound having the empirical formula $C_{12}H_{16}N_4SO_2$.

Further studies on the chemical constitution of this substance will be reported later on.

The author expresses his sincere thanks to Prof. Dr. U. Suzuki for his kind advise and encouragement throughout the work. Thanks are due to Sankyo Company, Ltd. for kind supply of the material. The author is also indebted to Messrs M. Kamada and T. Yamagishi for their assistance both in chemical and biological experiments.

(May 12 th., 1932).

Feeding Experiments with Decomposition Products of Proteins.

By

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(Received July 22, 1932.)

It is now generally assumed that protein in food is in a greater part hydrolysed in the alimentary canal into amino acids and again regenerated to the body protein, specific to each individual organism, so it is very probable that food protein can be substituted by the mixture of amino acids, provided that all necessary amino acids are present in adequate proportion. Abderhalden[1] was the first who tried to settle this problem by feeding experiment. In one experiment with a dog fed on the enzymatic decomposition products of lean meat, prepared by treating with pepsin, trypsin and erepsin successively, he has demonstrated that nitrogen equilibrium was maintained for many weeks. In another experiment with a dog, previously starved and lost a greater part of the body weight, he has observed that by giving the same decomposition products, the animal not only recovered the health but the body weight attained higher than before experiment. In a later period, he gave the mixture of amino acids to the same dog and observed that the nitrogen balance was still maintained, though for a short time.

U. Suzuki[2] reported also the positive results obtained with white rats fed on the synthetic diets containing either pepton or erepton. But he could not obtain any satisfactory results with the acid or baryta hydrolytic products. In this experiments a portion of meat protein was boiled with 25% sulphuric acid for 20 hours, sulphuric acid was carefully removed by baryta and evaporated to dryness. Another portion of meat protein was boiled with saturated baryta solutien for 6 hours and after removing baryta with sulphuric acid, evaporated to dryness. By adding these decomposition products, either alone or in combination, to the extent of 10% of the protein-free synthetic diet, otherwise adequate for growth, the rats could not maintain the health and gradually lost their body weights until finally succumbed.

Mitchell[3], McClendon[4] and Rose[5] have recently reported the results of

(1) Abderhalden: Z. physiol. Chem., **42**~**83** (1904~1913).
(2) U. Suzuki: J. Chem. Soc. Tokyo., **41**, **5** (1920), 381.
(3) Mitchell: J. biol. Chem., **26** (1916), 231.
(4) McClendon: Proc. Soc. Exper. Biol. & Med., **28** (1930~1931), 915.
(5) Rose: J. biol. Chem., **92** (1931), ixvi.

the similar experiments with mice and white rats fed on the synthetic diet containing the mixture of all known amino acids. The rats remained healthy for a month keeping their original body weights, but afterwards gradually declined.

We see thus that further experiments on this subject are desirable. From such a point of view, the present author has carried out some preliminary experiments with white rats, supplying the diets, in which the whole of the protein has been replaced by its partial or complete decomposition products.

In the first place, the enzymatic digestion products, such as Witte's peptone and Teruuchi's peptone were used and these were proved to be capable of replacing the dietary protein completely.

In the next experiments with the sulphuric acid and the baryta hydrolytic products of various degrees it was shown that neither the acid products alone, nor those by baryta could give any satisfactory results, but the mixture of the both was proved to be able to substitute the protein completely.

It is will known that the tryptophane in the protein molecule is completely destroyed by boiling with strong sulphuric acid, but it is not decomposed by baryta, though it undergoes racemization. Further it has been confirmed by several authors that tryptophane is one of the indispensable amino acids for building up the body protein, and it can not be substituted with any other amino acids. Taking these facts in consideration, the auther has carried out further experiments in which the acid hydrolysates were supplemented with tryptophane, instead of the baryta hydrolysates and came to the conclusion that tryptophane is the only factor which is lacking in the acid hydrolysates. By supplying it to the extent of 3% to the latter, the rats could secure the normal growth just like in the case, when the baryta hydrolysates were supplied.

Further experiments with the mixture of purified amino acids are now going on.

Experimental.

I. Experiments with digestion products by enzymes.

Commercial Witte's peptone (the pepsin digest of fibrin) and Teruuchi's peptone (the trypsin digest of casein) were used in this experiments, in the latter case 1% of cystine being added. The both are the mixtures of proteose and peptone, giving a specific biuret reaction. The analysis gave the following results.

	N%	Amino-N%			
		Van Slyke's Method		Formol method	
		Subst. %	N%	Subst. %	N%
Witte's peptone	16.28	1.69	10.39	1.13	6.94
Teruuchi's peptone	13·23	3.81	28.80	4.31	32.58

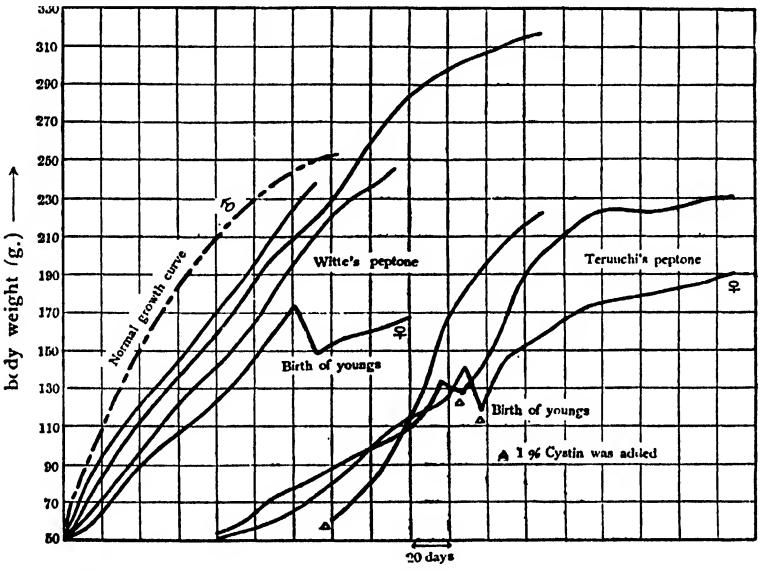


Fig. I.

Young albino rats were fed on the ration consisting of:

Each peptone	10%	Starch	70%
Butter	15%	McCollum's salt mixture	5%
Oryzanin	5 cc per 100 g.		

In both cases the rats grew normally and increased in body weights from 50 ~60 g. to more than 200 g. in 3 months (Fig. I).

II. Experiments with the acid and the baryta hydrolytic products.

(1) Experiments with hydrolytic products of fish meat protein.

The meat protein from codfish (total N 16·11% by Kjeldahl's method) was boiled with 6 times its quantity of 25% sulphuric acid for 30 hours, until it gave no biuret reaction, and showed no more increase of amino-nitrogen when boiled further for an hour. The second and the third portions were hydrolysed partially for one and ten hours respectively, besides the forth one was digested with 70% sulphuric acid at room temperature for 1 week, until

the greater part of the protein was converted into peptone[6].

Another portion of the fish meat protein was boiled with 6 times its quantity of hot saturated baryta* solution for 60 hours. The hydrolysate was free from biuret reaction, and showed no change in amino-nitrogen value when boiled further for one hour. Still other two protions were hydrolysed partially by baryta for one and ten hours respectively.

Sulphuric acid or baryta in each hydrolysed solution was quantitatively removed by baryta or sulphuric acid respectively, each filtrate from barium sulphate was neutralized with dilute sodium hydroxyde solution and then evaporated to dryness at lower tempesature. In each case 85～95 g. of the hydrolytic products were obtained from 100 g. of the protein. The analysis of these products gave the following results.

Condition of decomposition	Biuret reaction	N%	Amino-N %				Rotation		
			Van Slyke's method		Formol method		Subst. g. in 100 c.c. H_2O	α	$[\alpha]_D^{20°}$
			Subst %	N%	Subst %	N%			
by 70% H_2SO_4 at room temp.	+	15.99	4.62	28.93	2.47	15.42	3.2413	−1.65	−50.93
by 25% H_2SO_4 for 1 hr.	+	16.02	7.93	49.48	6.46	40.35	3.0727	−0.68	−22.13
" , " 10 hrs.	−	16.02	11.71	73.11	10.82	67.56	3.2084	+0.31	+ 9.67
" . " 30 hrs.	−	15.95	13.01	83.43	12.91	80.95	2.9435	+0.31	+10.53
by baryta, for 1 hr,	+	14.55	12.16	83.58	12.03	82.68	3.0861	0.00	0.00
" , " 10 hrs.	−	14.43	13.60	94.28	13.33	92.32	3.1411	0.00	0.00
" , " 30 hrs.	−	14.33	14.05	98.00	13.55	94.58	3.6026	0.00	0.00

Millon's, xanthoproteic and Pauly's reaction were positive in all of the above products. Ehrlich's tryptophane reaction by *p*-dimethyl-aminobenzaldehyde was positive with all of the baryta hydrolytic products. With the decomposition products by 70% sulphuric acid and those by 25% sulphuric acid for 1 hour the reaction was slightly positive, while the remaining two acid hydrolytic products gave entirely negative results. Sulphur reaction and the Sakaguchi's reaction for arginine were distinctly positive with the acid hydrolysates, but were entirely negative with the baryta hydrolysate.

Jaffe's rection for amino acid anhydride by picric acid and sodium carbonate were positive, except with the baryta hydrolysate and the 25% sulphuric acid hydrolysate for 30 hours.

From these colour reaction, it can be seen that in the acid hydrolysis,

(6) Fischer & Abderhalden: B., **39** (1906), 752.

* Baryta was recrystallized carefully.

tryptophane was destroyed, while in the baryta hydrolysis, cystine and arginine were decomposed, besides, the presence of amino acid anhydrides, in the partial hydrolytic products by sulphuric acid was indicated. Moreover, it can be seen that the protein loses its optical activity within an hour, when boiled with baryta.

Feeding experiments were carried out with the diets consisting of

Acid hydrolytic products	8%	Butter	15%
Baryta hydrolytic products	8%	McCollum's salt mixture	4%
Starch	65%	Oryzanin	5 c.c. per 100 g.

The combination of the hydrolytic products was as follows:

	Acid hydrolytic products prepared by:		Baryta hydrolytic products prepared by:
I.	70% H_2SO_4 at room temperature	+	Baryta for 1 hour.
II.	25% H_2SO_4 for 1 hour	+	Baryta for 1 hour.
III.	25% H_2SO_4 for 10 hours	+	Baryta for 10 hours.
IV.	25% H_2SO_4 for 30 hours	+	Baryta for 60 hours.

Besides, a diet of the following composition, containing the fish meat protein was used for the control.

Fish meat protein	10%	McCollum's salt mixture	5%
Starch	70%	Oryzanin	5 c.c. per 100 g.
Butter	15%		

Rats fed on the above diets grew healthy and increased in weight from 40 ~50 g. to 130~160 g. in two months, though the rate of growth was much slower than that of the control animals (Fig. II).

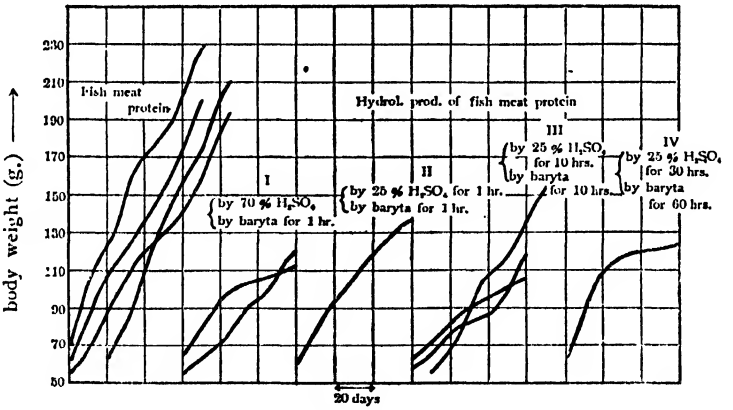


Fig. II.

The fact were worthy of note, that the products hydrolysed by sulphuric acid for 30 hours and those by baryta for 60 hours were free from biuret reaction, and showed, only slight increase in amino-nitrogen value, when boiled further for 5 hours with 20% HCl, as shown in the following example.

(i) The hydrolytic products of fish meat protein (by 25% sulphuric acid for 30 hours), were boiled further for 5 hours with 20% HCl, and the amino-N was estimated as follows:

	Amino-N %			
	Van Slyke's method		Formol method	
	Subst. %	N%	Subst. %	N%
Before boiling with HCl	13.25	83.31	12.91	81.00
After " " "	13.58	85.19	12.91	81.00
Increase	0.33	1.88	0.00	0.00

(ii) The hydrolytic products of fish meat protein (by hot saturated baryta solution for 60 hours), were boiled further for 5 hours with 20% HCl, and analysed as follows:

	Amino-N %			
	Van Slyke's method		Formol method	
	Subst. %	N%	Subst. %	N%
Before boiling with HCl	14.06	98.10	13.47	94.00
After " " "	14.29	99.81	13.75	95.92
Increase	0.23	1.71	0.28	1.92

Feeding experiments were repeated with the mixture of the above hydrolytic products i.e. with the mixture IV. As the source of vitamin B, first oryzanin and then the alcohol extract of yeast were used. The yeast extract was prepared acording to Williams and Watermann[7]. The extract itself or its precipitable part by phosphotungstic acid gave no biuret reaction. 100 c.c. of this extract yielded 12.4875 g. of dry matter and 0.7624 g. of nitrogen, the percentage of nitrogen in the dry matter being 6.11%. No noticeable increase was observed by boiling with 20% HCl for 5 hours more, indicating that almost all of the nitrogen were present in non-peptide form.

	Amino-N %			
	Van Slyke's method		Formol method	
	Subst. %	N%	Subst. %	N%
Before boiling with HCl	3.85	63.00	4.65	76.03
After " " "	3.86	63.24	4.68	76.60
Increase	0.01	0.24	0.03	0.57

(7) Willams & Watermann: J. Biol. Chem., **86** (1930). 275.

Two rats fed on the above diet grew normally from 56 g. and 62 g. up to 330 g. and 230 g. respectively in 8 months (Fig. III, VII, VIII, IX).

(2) Experiments with decomposition products of casein.

Similar experiments were carrid out with the biuret free decomposition products of casein by sulphuric acid and those by baryta. Rats grew from 62 g. and 73 g. to 91 g. and 100 g. respectively in 2 months, though the results were less satisfactory than the preceding experiments (Fig. III).

(3) Experiment with decomposition products of horse meat protein.

To the rats, previously fed on the casein decomposition products for 2 months, the mixture of the biuret-free hydrolytic products of horse meat protein by sulphuric acid and those by baryta were given. Rats grew vigorously as in the case with the hydrolytic products of the fish meat protein, and increased in weight up to 192 g. and 220 g. in 2 months (Fig. III, X, XI).

In the control experiment giving a protein free diet, the rat died within 20～25 days with a remarkable loss of weight. By giving water only without diet, the rats died within 2～3 days (Fig. III). The protein free diet referred to above had the following composition.

Starch	75%	McCollum's salt mixture	5%
Butter*	20%	Oryzanin	5 c.c. per 100 g.
Total N of starch :	0.04%	Total N of buttter :	0.09%

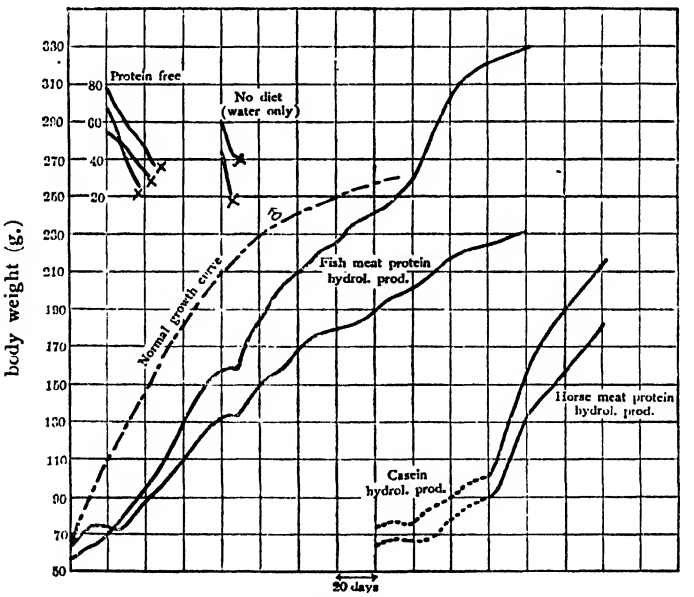


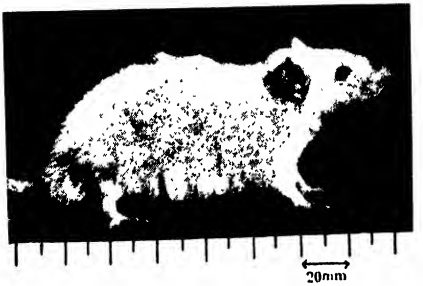
Fig. III.

The butter used in the experiment was extracted with ether and the ether-soluble portion was evaporated off.

Experiments with decomposition products of fish meat protein.

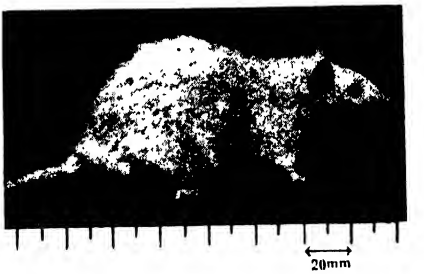
56 g. 62 g.

Fig. VII, Before the experiment.



330 g.

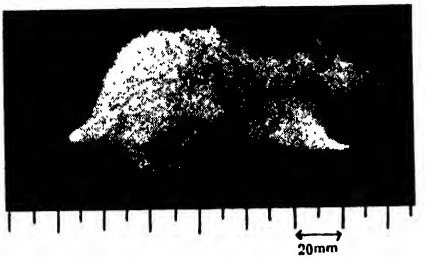
Fig. VIII. After 8 months.



231 g.

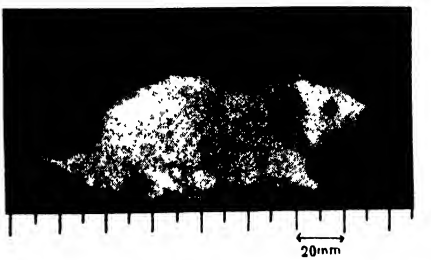
Fig. IX. After 8 months.

Experiments with decomposition products of horse meat protein.



192 g.

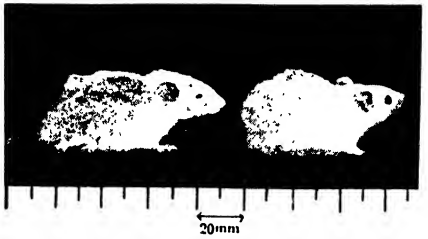
Fig. X. After 2 months.



220 g.

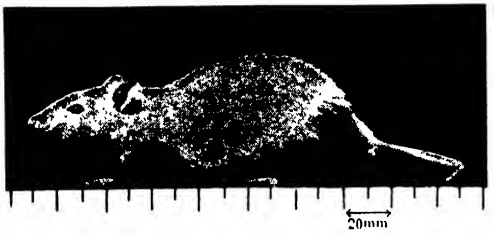
Fig. XI. After 2 months.

Experiments with acid hydrolytic product Supplemented with racemic tryptophane.



64 g. 65 g.

Fig. XII. Before the experiment.



187 g.

Fig. XIII. After 70 days.

190 g.

Fig. XIV. After 70 days.

Experiment with acid hydrolytic product of fish meat protein.

40 g.

Fig. XV. After 30 days.

III. Experiments with the acid and the baryta hydrolytic products separately.

The acid and the baryta hydrolytic products, complety free from biuret reaction were prepared from fish meat protein, as well as from horse meat protein, in the same manner as described before. Young rats were fed first on the diet consisting of:

Fish meat protein	15%	Starch	65%
Butter (ether soluble part)	15%	McCollum's salt mixture	5%
Alcohol extract of yeast	5%		

and let grow for 8 days. When the whole of the protein was replaced by each hydrolysate, the animals soon began to decline in body weights, and finally succumbed (Fig. IV, XV).

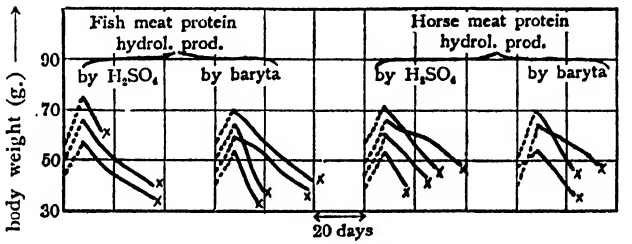


Fig. VI. Experiments with the acid or the baryta hydrolytic products.
------ fish meat protein.
——— hydrolytic product of protein.

IV. Experiments with the acid hydrolytic products supplemented with tryptophane.

In the first place, the tryptophane contents of the fish meat protein and its hydrolytic products were estimated according to Matsuyama and Mori's method[8], the tryptophane content of pure casein being assumed to be 1·5%

The results obtained were as follows:

Fish meat protein	1.24%	Acid hydrolytic products	0.00
Baryta hydrolytic products	1.78%		

From these results, it can be seen that tryptophane is completely decomposed by boiling with acid, but it is not destroyed, by treating with baryta. Intending now to decide whether the indispensable factor in the baryta hydrolytic product, which is lacking in the acid hydrolytic products, is tryptophane only the baryta hydrolytic products were fractionated by mercuric sulphate into two parts; i.e. the one, consisting solely of crystalline tryptohane and the other, the filtrate from $HgSO_4$-preciptate containing no tryptophane.

(8) Matsuya & Mori: J. Chem. Soc. Jap., **44** (1923), 377.

Mercury and sulphuric acid in the filtrate were removed by hydrogen sulphide and baryta respectively, the resulting filtrate was neutralized with dilute caustic soda, and then evaporated to dryness at lower temperature. From 200 g. of fish meat protein, 1.5 g. of crystalline tryptophane and 130 g. of the filtrate part were obtained.

Tryptophane obtained as above stated was optically inactive, and gave the following analytical result.

		N%
Subst. 3.750 mg.	0.439 c.c. N (765.7 mm, 21.5°)	13.67
3.435 mg.	0.400 c.c. N (765.8 mm, 22.0°)	13.56
Calculated for tryptophane:		13.72

Rats were fed first on fish meat protein and let grow for 8 days, then the protein was replaced by its acid hydrolysate, so the rats began to lose their body weights, owing to the lack of tryptophane. Then two rats were supplied with racemic tryptophane* to the extent of 3% of the acid hydrolysate, and to the other two rats the filtrate part of the baryta hydrolysate was given repalacing half the quantity of the acid hydrolysate. Hereupon, a sudden increase of body weight was resulted in the former case namely on the ration containing tryptophane. Rats grew normally from 64 g. and 65 g. up to 187 g. and 190 g. respectively in 70 days, while the rats receiving the latter ration lost their body weights remakably. To the remaining one, supplied with tryptophane, and grown vigorously for 38 days was given the baryta hydrolysate, replacing half the quantity of the acid hydrolysate, tryptophane being omitted. In this case also, the rat soon began to decline in body weights (Fig. V, XII, XIII, XIV). From these results, it may be seen that the filtrate part of the baryta hydrolysate has no supplementing effect on the acid hydrolysate, but, what should give us concern is that some toxic substance might contaminate the filtrate during the process of fractionation with mercuric sulphate, so the experiments were repeated with the following scheme:

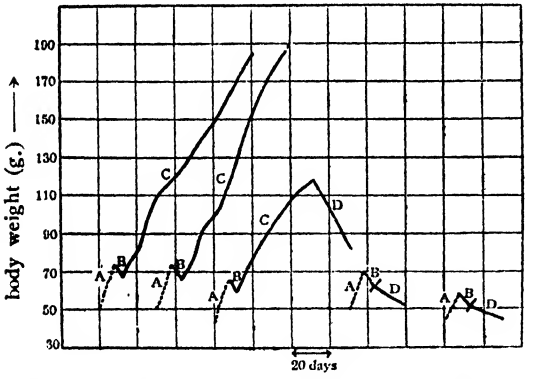


Fig. V. Experiments with acid hydrolytic product supplemented with tryptophane.
A: Fish meat protein; B: Acid hydrol. prod.
C: B. suppl. with tryptophane (3% of the acid hydrol. prod.)
D: B. suppl. with the filtrate part in the baryta hydrol. prod.

* 20 g. of racemic tryptophane was prepared from 2.5 kg. of casein by boiling with baryta.

Rats were fed first 20 days on the diet consisting of:

Acid hydrolytic products	7.5%	Butter (ether soluble part)	15.0%
Baryta hydrolytic products	7.5%	McCollum's salt mixture	5.0%
Starch	65.0%	Alcohol extract of yeast	5.0%

After 20 days, when the baryta hydrolytic products were replaced by its tryptophane-free filrate part, the growing rats soon began to loose their weights remarkable. By adding tryptophane (3% of the hydrolytic products) the rats revived suddenly and began to grow vigorously. After 20 days, the filtrate part was removed from the above diet but no effect on growth was observed and the rats continued to grow normally as before (Fig. VI). From these results, the author wishes to conclude that the acid hydrolytic products of protein, when supplemented with tryptophane, can replace the protein in diets completely.

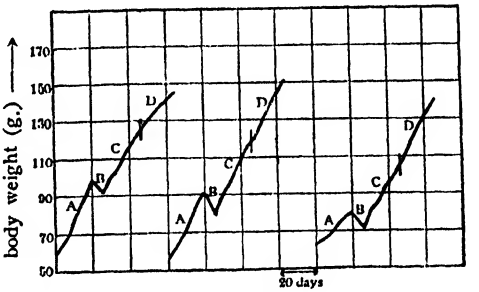


Fig. VI. Similar experiments as shown in Fig. II.

A: Mixture of the acid and the baryta hydrol. prod.

B: Acid hydrol. prod. and the filtrate part in the baryta hydrol. prod.

C: B. suppl. with tryptophane (3% of the hydrol. prod.)

D: Acid hydrol. prod. suppl. with 3% of tryptophane.

In conclusion, the author wishes to expess his deepest obligations to Prof. U. Suzuki for his kind directions throughout this work.

Carotin in Mango Fruit (*Magnifera indica* Lin.).

By

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(Received August 16, 1932.)

Works of H. Euler, P. Karrer[1][2][3], K. Kawakami[4], H. Mattil[5], R. Kuhn[6], and their co-workers to the effect that carotin has a function of vitamin A gave the significant contribution in the study of food chemistry. The colouring matter of water-melon[7] and tomato[8] has more or less to do with carotin, so that the occurrence of carotin in other fruits which contain yellow pigment might be reasonably suspected. The fruit pulp of mango is of well-known yellow colour, and if this pigment belongs to carotin, it may give interesting information to the nutritive value of mango fruit.

Mango fruit, we have got, was the native variety produced in Formosa. According to M. Isii the fresh pulp of the fruit contains 81.7% water, 3.0% citric acid, 1.5% glucose, 4.9% fructose, 5.5% sucrose, 0.5% crude fiber, 1.3% ether extract, 0.1% nitrogen, and 0.5% ash.

The yellow pigment is easily soluble in ether, pet. ether, chloroform, and partly soluble in alcohol, but not in water. The colour reaction by antimony trichloride is blue. These behaviours suggested the existense of a certain carotinoid. The isolation was carried out according to Willstätter, Kuhn and Karrer.

Main obstacles for isolation were the presence of waxy substance mixed with the pigment and moreover, as the fruits are produced only in hot summer time, the treatment of a large amount at one time was somewhat cumbersome. The details of the experiment will be found in the experimental part. We could isolate pure carotin in crystalline form, but the complete separation[9] of α- and β- carotin were not attempted owing to the paucity of the material, only the general separation was carried out by the fractional crystallization from carbon disulphide, ethyl alcohol and pet. ether.

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(1) Helv. Chim. Acta, (1929), 278.
(2) Helv. Chim. Acta, (1931), 831.
(3) Helv. Chim. Acta, (1931), 1036, 1431.
(4) Proc. Imp. Acad. Jap., (1929), 213.
(5) J. Biol. Chem., (1931), 1050.
(6) B., **64** (1931), 1859.
(7) B., **63** (1930), 2881.
(8) Helv. Chim. Acta, (1931), 154.
(9) Hoppe Sy. Z. Physiol. Chem., **200** (1931), 246.

The main fraction melted at 179° and specific rotation was $[\alpha]_C^{28°} = +136°$. These indicated the fraction to be a mixture of α and β carotin, the absorption spectrum also proving it. In addition to this main fraction a little quantity of crystal, melting at 174° was separated. It was more soluble in pet. ether and was ascertained to be α-carotin. The yield of main fraction was 0.06 g. from 38 kg. of fresh mango fruit.

A quantitative estimation of carotinoids in mango fruit was determined according to Kuhn and Brockmann[10]. The result was 0.1179 g. carotin, 0.0785 g. xanthophyll in ester form, and 0.0156 g. free xanthophyll, in 1 kg. anhydrous pulp of mango fruit.

The isolation of xanthophyll in crystalline form was unsuccessful, so it was determined by absorption spectrum, and the result coincided with the blattxanthophyll[11].

It seems from the experiment that the content of carotinoids is varied by the variety and the ripeness of the fruit.

The physiological action of the carotin as vitamin A was determined by the feeding experiment which was kindly carried out by K. Kawakami in the Institute of Physical and Chemical Research in Tokyo.

The daily doses of 0.05 mg. of the carotin (m. p. 179°) was quite sufficient for curing of albino rats from their deficiency of vitamin A.

Experimental*.

Isolation of carotin:

The fresh pulp of mango fruit (38 kg.) was mashed, and extracted with ether three times. The ether was then washed with water, dried and distilled in the atmosphere of carbon dioxide. When the ether was evaporated completely by vacuum, a yellowish red oily substance was obtained. This was then dissolved in pet. ether, washed by 90% methyl alcohol several times to get rid of xanthophyll.

When the pet. ether solution was dried with sodium sulphate, evaporated to a small quantity and left in an ice chest for 48 hours, it subsided colourless needles mixed with waxy substance. The filtrate from the above was then saponified by 2 N alcoholic potash in nitrogen atmosphere on water-bath during two hours. By this treatment, carotin crystallized out slowly, which was collected and washed first with water and then with methyl alcohol. The crude carotin thus obtained was dissolved in a little quantity of carbon disulphide and by the addition of absolute alcohol fractionally precipitated.

(10) Hoppe Sy. Z. Physiol. Chem., **206** (1932), 41.
(11) " " " " " **197** (1931), 141.

* The experiment was carried out in each summer of 1930 to 1932.

When the filtrate from the first crop was cooled in an ice chest a second fraction was obtained, and third fraction was separated by adding absolute alcohol to the above filtrate. The each fraction was recrystallized from pet. ether. We could, thus, separate two kinds of crystal of different melting point. The main fraction melted at 179°, and a very small part melted at 174°, both of which showed a deep red metallic lustre, the former being long prisms and the latter rhombic plates.

Analysis of carotin:

1) Carotin m. p. 173—174°.

Substance (mg.)	CO_2 (mg.)	H_2O (mg.)	C %	H %
1.891	6.203	1.791	89.46	10.60
1.090	3.583	1.033	89.65	10.61
Calc. for $C_{40}H_{56}$			89.55	10.45

2) Carotin m. p. 179°.

Substance (mg.)	CO_2 (mg.)	H_2O (mg.)	C %	H %
1.436	4.699	1.340	89.25	10.44
1.834	12.548	3.535	89.26	10.32
Calc. for $C_{40}H_{56}$			89.55	10.45

Absorption spectrum:

1) Absorption spectrum of α-carotin (m. p. 173—174°) by quartz, spectrograph in chloroform solution, absorption bands;

I 452—487 $\mu\mu$, II 495—507 $\mu\mu$.

2) For the main fraction (m. p. 179°) the maximum absorption was observed by spectro-photometer in CS_2 and chloroform solution.

Absorption bands

	I	II	III
In CS_2	449 $\mu\mu$	475 $\mu\mu$	514 $\mu\mu$
In chloroform	435	462	487

Specific rotation:

The optical rotation of the main fraction m. p. 179° in CS_2;

$[\alpha]_C^{28°} = (+0.15 \times 100) : (1.5 \times 0.7232) = +135°$.

Estimation of carotinoids in mango fruit:

320 g. fresh pulp from 574 g. fruit (five) was mashed and from this homogeneous mass 60 g. was taken and treated with methanol and then methanol pet. ether mixture, until all the pigment was disappeared. Then diluted with water and extracted by pet. ether, filled up to 500 c.c.. From

this solution 100 c.c. was taken and estimated carotinoids according to Kuhn and Brockmann[10].

Mean reading of carotin solution corresponds to 10 mm.

standard solution ..... 4.6 mm. ..... total solution 250 c.c.
" " free xanthophyll " "
standard solution ..... 15.5 mm. ..... total solution 100 c.c.
" " ester form xanthophyll " "
standard solution ..... 7.7 mm. ..... total solution 250 c.c.

Carotin in 12 g. fresh pulp
$= 0.0047 \times 10/4.6 \times 250 = 0.245$ mg.,
free xanthophyll in 12 g. fresh pulp
$= 0.000504 \times 10/15.5 \times 100 = 0.0325$ mg.,
ester form xanthophyll in 12 g. fresh pulp
$= 0.000504 \times 10/7.7 \times 250 = 0.163$ mg.

	Carotinoids in 1 kg. pulp mango fruit	
	Fresh pulp (water content 82.7%)	Anhydrous pulp
Carotin	20.41 mg.	0.1178 g.
Free xanthophyll	2.70	0.0156
Ester xanthophyll	13.58	0.0780

Absorption maximum of xanthophyll fraction in CS_2 by spectrophotometer.

	Absorption bands		
	I	II	III
Free xanthophyll	506 $\mu\mu$	473 $\mu\mu$	446 $\mu\mu$
Ester form xanthophyll	506—505	475	445

On the Carotinoids in Fresh Tea-Leaf and Fermented Tea.

By

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(Received August 8, 1932.)

The colouring matters of fermented tea such as black and Oolong tea

consist of water soluble and insoluble parts, and the former is mainly tannic material and the latter is suggested to be carotinoids and chlorophyll. In addition to the study of tannin in fresh tea-leaf and fermented tea, the preparation of which is now carried on in our laboratory, we have investigated the carotinoids.

The materials, we have taken, were Formosan tea-leaf and its fermented tea. The fresh tea-leaves, and fermented tea-leaves, which were taken from fermenting room in the factory, were dried at 40° and powdered. This material was extracted with ether, the ethereal solution was then concentrated and saponified with alcoholic potash; this was diluted with much water and again extracted by ether, which was then washed with water, dried and evaporated ether completely. The red colouring matter thus obtained was separated into carotin and xanthophyll by means of pet. ether and 90% methyl alcohol.

Xanthophyll.

The methyl alcoholic solution separated from pet. ether, was diluted with water until 70% concentration, shaken with pet. ether, and left standing in an ice-chest. After 24 hours, xanthophyll crystallized out between two layers. It was dissolved in little chloroform and recrystallized by adding pet. ether, and finally recrystallized several times from methyl alcohol. The purified melting substance melted at 192°. From black tea, Oolong tea and Touchang tea we have separated xanthophyll of the same melting point. The absorption bands of spectrum was on 504 $\mu\mu$, 473 $\mu\mu$, 446 $\mu\mu$, and the specific rotation was $[\alpha]_C^{20°} = +170°$ (in acetic ester). These results as elemental analysis agreed with those of blattxanthophyll[11].

Carotin.

When the pet. ether solution mentioned above, was evaporated in vacuum, added with absolute alcohol and cooled in an ice chest, carotin was separated. Thrice recrystallized from benzene and methanol, deep red crystals of carotin (m. p. 174°) were obtained. Carotin can also easily be obtained by treating the above pet. ether extract with acetone before the separation of xanthophyll; but such treatment makes the isolation of xanthophyll rather difficult. The maximum absorption of spectrum was on 510—511 $\mu\mu$, 477 $\mu\mu$, 435 $\mu\mu$ (in CS_2). Specific rotation was $[\alpha]_C^{20°} = +370°$ (in CS_2). These experimental data and the results of elemental analysis have just agreed with α-carotin of Kuhn[2][3]. We had also isolated carotin in crystalline form from

(1) Kuhn and Winterstein: Hopp. phys. Chem., **197** (1931), 141; **63** (1930), 1489.
(2) Kuhn: Naturwissenschaften, **306** (April) (1931).
(3) Kuhn and Brockmann: **64** (1931), 1859.

Oolong tea, Touchang tea, but could not from black tea, so we had confirmed its presence in the latter by the spectrum analysis.

The estimation of carotinoids was carried out by the method of Willstätter and Stoll[4].

The results were:

Carotinoids in 1 kg. materials (anhydrous)

	Carotin (g.)	Xanthophyll (g.)
Fresh leaf	0.1751	0.4544
Fermenting tea-leaf	0.1571	0.4237
Touchang tea	0.1681	0.4257
Oolong tea	0.0784	0.2441
Black tea	0.0715	0.3686
Green tea	0.1571	0.4175

As for carotin in green tea Miss Tsujimura[5] has recently isolated it in crystalline form, our experiment had been accomplished before her publication.

Elemental analysis of carotinoids.

(1) *Xanthophyll*:

Xanthophyll separated from both fresh leaf and fermented tea had the same melting point 192°. The analytical results were:

Substance (mg.)	H_2O (mg.)	CO_2 (mg.)	H%	C%
5.304	4.671	16.435	9.86	84.51
2.550	2.211	7.887	9.73	84.35
Calc. $C_{40}H_{56}O_2$ required			9.93	84.44

(2) *Carotin*:

Carotin in fresh leaf and fermented tea had the same melting point 174°.

Substance (mg.)	H_2O (mg.)	CO_2 (mg.)	H%	C%
3.514	3.225	11.436	10.37	88.77
Calc. $C_{40}H_{56}$ required			10.52	89.48

Sepecific rotation.

Xanthophyll m p 192°.

in acetic ester $[\alpha]_C^{23} = (+ 0.02° \times 100) : (1 \times 0.0117) = + 170°$

(4) S. Palmer: Carotinoids and Related Pigment, 248.

(5) Tsujimura: Sc. Pap. I. P. C. R., Tokyo., **349** (1932).

in CS_2 ..... $[\alpha]_C^{20} = (+ 0.04° \times 100) : (1 \times 0.0117) = + 341°$

Carotin m p 174°.

in CS_2 ..... $[\alpha]_C^{23} = (+ 0.13° \times 100) : (2 \times 0.0184) = + 353°$

Absorption spectrum.

The extinction coefficient had been measured using Adam-Hilger's spectro-photometer and maximum absorption were observed both crystalline carotin and xanthophyll in CS_2 solution (Mol $\times 10^{-5}$ conc.) (Fig. 1).

Maximum absorptions were,

Carotin	510~511 $\mu\mu$	476~479 $\mu\mu$	434~435 $\mu\mu$
Xanthophyll	504	473	446

For the determination of carotin in black tea, spectroscopic analysis had been applied in carotin fraction (in pet. ether sol.) which was employed for colourimetric measurement. The standard solution in this case was pure α-carotin in pet. ether.

Maximum absorptions of carotin fraction in black tea (in pet. ether sol.)

426 $\mu\mu$ 448 $\mu\mu$ 474 $\mu\mu$

of standard pure α-carotin solution (in pet. ether sol.)

425 $\mu\mu$ 446 $\mu\mu$ 474 $\mu\mu$

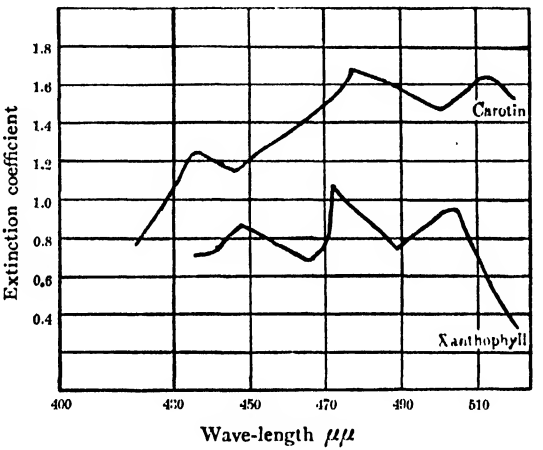

Fig. 1.

Quantitative analysis of carotinoids.

In both xanthophyll and carotin 14 g. materials were taken and analyzed according to Willstätter and Stoll[4]. For the standard solution 0.0013 g. pure carotin in 100 c.c. pet. ether and 0.0028 g. pure xanthophyll in 100 c.c. ether were employed. The concentration of the standard solution was $5 \times 10^{-5} \times 1/2$ Mol.

0.1% aqueous potassium bicromate was also used as the standard for xanthophyll.

100 mm. of xanthophyll standard solution

= 81.0 mm. potassium bicromate solution.

In each solution, colour intensity was compared with standard solution 5 mm. to 45 mm., and mean value was observed, and this converted into 100 mm. The result was,

The reading of colourimeter in mm.

	Carotin		Xanthophyll	
	Water content %	Carotin (standard) 100 mm.	Water content %	$K_2Cr_2O_7$ 100 mm.
Fresh leaf	55	243.6	55	195.1
〃	7	110.3	7	97.7
Fermenting tea-leaf	52	261.4	52	208.0
〃	12	134.6	12	191.6
Black tea	7	267.6	7	106.3
〃	6	307.6	6	132.0
				Standard pure xanthophyll
Oolong tea	6	374.2	6	189.1
Touchang tea	11	127.4	11	106.6
Green tea	5	224.6	6	179.2

Calculation of carotin and xanthophyll gave,

carotin $50 \times 0.536 \times 1/2 \times 1/2 \times 100/C \times 2.857$ g.

(the original solution was diluted twice its volume in this case)

xanthophyll $50 \times 0.568 \times 1/2 \times 100/X \times 2.857$ g.

where, C = the reading of carotin solution equal to 100 mm. standard solution,

X = the same as above for xanthophyll 2.857 = dilution factor.

Carotin and xanthophyll contents in anhydrous 1 kg. material:

	Carotin (g.)		Carotin mean value	Xanthophyll (g.)		Xanthophyll mean value
Fresh leaf	0.1746	0.1757	0.1751	0.4623	0.4465	0.4544
Fermenting tea-leaf	0.1526	0.1617	0.1571	0.4063	0.4411	0.4237
Black tea	0.0769	0.0662	0.0715	0.4103	0.3269	0.3686
Oolong tea	0.7840			0.2411		
Touchang tea	0.1681			0.4257		
Green tea	0.1571			0.4175		

On the Organic Acids in the Fruits of Ceylon Olive

(*Elaeocarpus serratus* Lin.)

By

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(Received August 16, 1932.)

The fresh fruits and pickles of the fruits of Ceylon olive* have been eaten by Formosan and south Chinese from long time ago and still in present time.

The fruit has special sour taste and we have investigated its component. The main part of the acids was citric, besides which we have separated a crystalline acid, almost insoluble in water. It crystallized in prisms of m p 215° and it was identified as a mucic acid. Under microscope, we couldn't find any crystal in the fruit, but this crystal was easily observed when the fresh fruit had been boiled in water for twenty minutes and stored in ice-box for two days. These facts suggested that mucic acid must be in free state in the fruit.

Besides acid fractions we observed that the fresh juice indicated strong Benzzonow's Vitamin-C reaction. We have determined its content by the feeding experiment with guinea-pigs. Four cubic centimeters of the juice per day was the minimum quantity to maintain the normal growth of the animal, while in the case of lemon juice 3 c.c. per day was quite sufficient*.

Experimental.

To the pulp of fresh fruit, alcohol was added until 70% concentration to precipitate pectinic substance. The alcohol was then evaporated and the residue was neutralized with dilute sodium carbonate solution. The organic acid was then precipitated by lead acetate, and the lead salt was decomposed by sulphuretted hydrogen. The isolation of organic acids was carried out according to Schmidt. Citric acid was thus separated, a trace of tartaric acid was detected, but no oxalic, fumaric, and succinic acid was traced. Besides these acids, crystals of m p 215°, almost insoluble in cold water, were obtained, but the yield was very scanty.

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* The composition of the pulp of the fruit was:

Water	85.73%	Ash	0.62%
Total acid (as citric acid)	4.37	Crude fiber	1.80
Reducing sugar (as glucose)	1.93	Crude fat	0.23
Non-reducing sugar (as sucrose)	0.98	Nitrogen	0.05

To obtain mucic acid only, alcoholic solution (50%) of mashed pulp was neutralized directly with barium hydroxide. The precipitate was then decomposed by sulphuric acid, and the filtrate was concentrated and cooled. After a while, mucic acid separated out. When recrystallized from boiling water, it forms prismatic crystals of m p 215°. The yield was 0.5% of the fruit.

Analysis of calcium citrate:

Substance (anhydrous)	CaO	Ca %
0.3604 g.	0.1272 g.	24.67
Cal. $(C_6H_5O_7)_2Ca_3$ required		24.13

Analysis of mucic acid:

Substance (mg.)	CO_2 (mg.)	H_2O (mg.)	C %	H %
9.205	11.480	3.952	34.01	4.80
6.778	8.401	3.020	33.81	4.98
Cal. $C_6H_{10}O_8$ required			34.29	4.76

Silver salt of mucic acid:

Substance (mg.)	Ag (mg.)	Ag %	Mol. weight
22.016	11.161	50.69	
14.675	7.431	50.64	209.88
Cal. $C_6H_8O_8Ag_2$ required		50.91	
Cal. $C_6H_{10}O_8$ "			210.08

Phenylhydrazide from the separated mucic acid melted at 238°, and was identified as pure mucic acid phenylhydrazide.

On the Red Colouring Matter of *Hibiscus Sabdariffa* L.

(A New Glycoside *Hiviscin.*)

By

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(Received August 16, 1932.)

In 1909 A. G. Perkin[1] isolated a yellow colouring matter *gossypetin* from

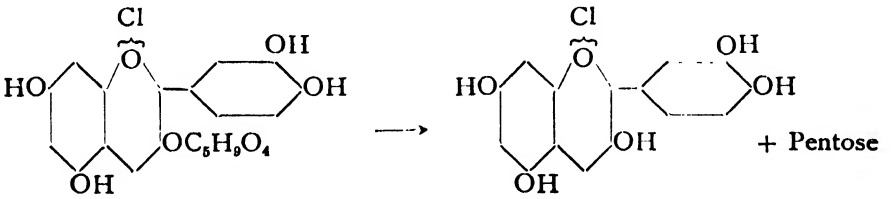
(1) A. G. Perkin: J. Chem. Soc., (1909), 1855.

the calyx of the fruit of *Hibiscus Sabdariffa* L. (Roselle), a small tropical shrub, and this gossypetin had been later synthesized by Baker, Nozu and Robinson[2]. The calyx is originally of a deep red colour and is easily suggested that the fruits contain originally red compound besides gossypetin.

The red colouring matter is easily soluble in water, it changes to fine red by hydrochloric acid and by alkali to bluish green, these indicating the presence of anthocyanin. So the investigation of the pigment has been carried out according to Willstätter, Kaller and Robinson.

The dried and powdered material was first extracted with 1.5% methyl alcoholic hydrogen chloride, the pigment was then precipitated by ether, and converted into picrate. When this picrate was decomposed by 7% methyl alcoholic hydrogen chloride, brownish red prisms (m p 192°) were obtained, which proved to be an anthocyanin chloride. This red colouring matter is soluble in water, with reddish violet colour, which changes into fine red by hydrochloric acid, into violet blue by sodium carbonate, and very unstable pure blue by sodium hydroxide. The result of oxidation test with ferric chloride showed that it belongs to cyanin group, and moreover, the distribution number between 0.5% hydrochloric acid and amyl alcohol indicated that the pigment is an anthocyanin monoxide[3].

The isolated anthocyanin chloride contained 4 $\frac{1}{2}$ mol crystalline water. The analytical result of anhydrous crystal was $C_{20}H_{19}O_{10}Cl$, and by hydrolysis it was confirmed that the anthocyanin consists of a cyanidin and one molecule of pentose :-



Hiviscin chloride Cyanidin chloride

As the separated anthocyanin was quite different from gossypetin and such an anthocyanin, which is combined with a pentose has not yet been found in nature, so we wish to give the name *hiviscin* to it.

The determination of the nature of the carbohydrate residue could not be made thoroughly on account of scarcity of the materials, but the colour reaction, optical properties and its derivatives confirmed that the carbohydrate must be a pentose.

As for the attaching position of the sugar to cyanidin, we had concerned

(2) Baker, Nozu and Robinson: J. Chem. Soc., (1929), 74.
(3) Robertson and Robinson: Biochem. J., (1931), 1690.

the oxidation test with ferric chloride according to Robinson[4], and the time of decoloration was almost coincided with that of idein and chrysanthemin, indicating that the pentose is probably attached to 3 position of the flavyl group.

The maximum absorption of the spectrum and the colour reaction in buffered solution were also described in the experimental part.

Experimental

Isolation of hiviscin chloride:

1.200 kg. dried calyx of the fruits were extracted with 3.6 L. methyl alcoholic hydrogen chloride (1.5%) in room temperature for about 48 hours, filtered on Buchner funnel and again extracted with 2 L. methyl alcoholic hydrogen chloride of the same concentration. After 24 hours, it was filtered and pressed, the total filtrate amounting to 5 L. To this alcoholic extract, 13 L. of ether were added, whereby the colouring matter was deposited as syrupy mass. It was dissolved in 800 c.c. of methyl alcoholic hydrogen chloride (1%) and again precipitated by the addition of 2.5 L. ether. The syrupy colouring matter was now dissolved in 500 c.c. hot water and was converted into picrate by means of 200 c.c. hot saturated aqueous picric acid. The resinous matter was first filtered and the filtrate was cooled in an ice-chest for one week. The crude picrate which crystallized out was collected and the filtrate was again concentrated in vacuum at room temperature (20°) and again cooled in ice-chest for one week, the deposited picrate being rather pure in this case. It was tried to recrystallize the crude picrate from 0.5% methyl alcoholic hydrogen chloride, but the resulted deep red prismatic needles were contaminated with free anthocyanin chloride and the yield was not good. The crude picrate was therefore converted into anthocyanin chloride directly by means of dissolving picrate (3.5 g.) in 2.0% methyl alcoholic hydrogen chloride (200 c.c.) and 800 c.c. of ether was added. After cooling this mixture for 24 hours in an ice-chest, the precipitated colouring matter was collected carefully. This was again dissolved in 0.5% hydrochloric acid (150 c.c.), and the filtrate from resinous matter was added with 7% methyl alcoholic hydrogen chloride (500 c.c.), cooled in ice-chest for 24 hours, when metallic brownish red needle crystal was obtained. After repeating this treatment once more, the anthocyanin chloride was almost pure, but again recrystallized from 1% methyl alcoholic hydrogen chloride m p 192°. The yield was 2 g..

Picrate 0.03 g. anthocyanin chloride was dissolved in 2 c.c. hot water and was added with 1 c.c. hot aqueous picric acid solution. Deep red needles (gathering) of m p 185° (dec.).

(4) Grove and Robinson: J. Chem. Soc., (1931), 2737.

The anthocyanin chloride thus odtained contains 4 ½ mol crystalline water, one mol of which was strongly combined. The anhydrous compound was obtained by drying it at 100° under 1 mm. pressure.

The results of analysis were;

Crystalline water:

0.1938 g. air-dried substance lost (at 100° in 1 mm.) water in weight 0.0265 g. H_2O 15.74%

Cal. for $C_{20}H_{19}O_{10}Cl \cdot 4\frac{1}{2} H_2O$ H_2O 15.23%

Air-dried substance:

Substance (mg.)	H_2O (mg.)	CO_2 (mg.)	H%	C%	Cl%
4.090	1.921	6.804	5.26	45.37	
4.925	2.179	8.200	5.19	45.41	
7.143	Rhodan titration 0.275 c.c.				6.51
Cal. for $C_{20}H_{19}O_{10}Cl \cdot 4\frac{1}{2} H_2O$			5.26	45.11	6.56

For the estimation of chlorine Ter-Meulen and Heslingas' method was applied, where 1 c.c. of rhodan solution = 1.900 mg. Cl.

Substance containing 1 mol H_2O:

0.1032 g. substance (dried at 100° in 20 mm.) lost 0.0044 g. in weight at 100° in 1 mm. H_2O 4.26%

Cal. for $C_{20}H_{19}O_{10}Cl \cdot H_2O$ H_2O 3.82%

Substance (mg.)	H_2O (mg.)	CO_2 (mg.)	H%	C%	Cl%
2.071	0.826	3.846	4.46	50.65	
3.596	1.446	6.718	4.48	50.96	
6.286	2.470	11.668	4.40	50.63	
3.132	1.238	5.834	4.42	50.80	
6.413	Rhodan titration 0.200 c.c.				7.45
Cal. for $C_{20}H_{19}O_{10} \cdot H_2O$			4.45	50.85	7.51

Anhydrous substance dried at 100° in 1 mm:

Substance (mg.)	H_2O (mg.)	CO_2 (mg.)	H%	C%	Cl%
2.151	0.835	4.153	4.34	52.66	
3.417	1.432	6.546	4.69	52.25	
7.153	Rhodan titration 0.285 c.c.				7.57
4.742	Rhodan titration 0.185 c.c.				7.41
Cal. for $C_{20}H_{19}O_{10}Cl$			4.18	52.86	7.71

Properties of hiviscin chloride:

Hiviscin chloride crystallizes in brownish red long prisms, but under

microscope it seems greyish violet. Its aqueous solution is violet, but the colour changes red by hydrochloric acid. The colouration in aqueous sodium carbonate is violet blue, but on addition of aqueous sodium hydroxide it turns blue, and quickly fades away into greenish yellow. In alcoholic solution hiviscin shows carmine red colour with violet nuance, but on addition of ferric chloride the colour becomes pure blue, which becomes violet when diluted with water. Further, by aqueous lead acetate the colour becomes blue violet and by alum violet, both of them giving stable colouration.

Oxidation test by ferric chloride:

Léon and Robinson[5] stated, that the time of decolourization of anthocyanin by ferric chloride is different according to the position of carbohydrate residue attached to flavyl group. According to them, for 3 glucoside in cyanin group, the colour is slowly oxidized, and in 1 hour 35 minutes the colour is nearly discharged, while for 5 glucoside (synthetic) colour was destroyed only in 10 minutes.

In the case of hiviscin, 2.020 mg. anthocyanin chloride was dissolved in 50 c.c. 1% hydrochloric acid. and after three minutes 50 c.c. of 0.125% ferric chloride was added. The colouring matter was gradually oxidized, and after 1 hour 40 minutes the colour was almost discharged.

Distribution number of hiviscin:

This was carried out by direct comparison method according to Levry and Robinson[5], using 0.5% hydrochloric acid (50 c.c.) and amyl alcohol (50 c.c.). The result was;

Substance (mg.)	in G. mol. $\times 10^{-6}$	Distribution number	
		1st shake	2 nd shake
13.00	26.82	15.20	15.38
6.50	13.41	16.61	16.64
3.25	6.69	16.92	17.08
1.30	2.68	17.48	18.12

Absorption spectrum of hiviscin and cyanidin:

The maximum absorption in water solution (10^{-5} mol) was observed using spectrophotometer.

$\mu\mu$	Cyanidin chloride	Hiviscin chloride	$\mu\mu$	Cyanidin chloride	Hiviscin chloride
600	0.29	0.38	555	0.46	0.60
590	0.33	0.43	550	0.42	0.59
580	0.35	0.59	545	0.41	0.58
575	0.38	0.62	540	0.40	0.58
570	0.43	0.62	530	0.38	0.54
565	0.46	0.61	520	0.36	0.46
560	0.48	0.61			

(5) Levry and Robinson: J. Chem. Soc., (1931), 2720.

(5) loc. cit.

Maximum absorption lies on 560 $\mu\mu$ and 570~575 $\mu\mu$ with cyanidin chloride and hiviscin chloride respectively.

Colour reaction in buffered solution:

The method of Robertson and Robinson[6] was employed and the numbers refer to solution of PH 3.2 (1) to PH 11.0 (14) and then to more alkaline solution.

1% and 20% hiviscin hydrochloric acid, stable eocin red.

(1) light pink, stable.

(2) almost the same as (1), but with slight violet nuance, after ten minutes, the colour became light red violet,

(3) violet pink.

(4) the same as (3).

(5) reddish violet.

From (3) to (5) fading into light violet pink after 30 minutes.

(6) reddish violet, deeper in violet than (5).

(7) the same as (6).

(8) light red violet.

These three fading to light red violet after 30 minutes.

(9) light red violet, but lighter than (8).

(10) light violet red.

(11) the same as (10).

(12) light violet.

These four fading more rapidly, after 30 minutes very light red violet.

(13) violet, faded very rapidly.

(14) the same as (13), faded very rapidly.

(15) bluish violet, faded very rapidly.

(16) light blue, faded very rapidly.

(17) light blue, faded very rapidly.

Hydrolysis of hiviscin chloride:

(I) 0.1606 g. anhydrous material was dissolved in 6 c.c. hot water, added with 6 c.c. concentrated hydrochloric acid and was boiled 2 minutes. After cooling the cyanidin chloride was filtered. It crystallized in deep red violet gathering needles: yield 0.0924 g. (anhydrous).

(II) 0.1430 g. anhydrous material was dissolved in 3 c.c. hot water, added with 3 c.c. concentrated hydrochloric acid and was boiled 2 minutes: yield of anhydrous cyanidin chloride was 0.0987 g.

Analysis of cyanidin chloride:

The material dried at 100° in 1 mm. pressure was analyzed.

(6) Robertson and Robinson: Biochem. J., (1929), 35.

Substance (mg.)	H_2O (mg.)	CO_2 (mg.)	H%	C%	Cl%
7.951	2.704	16.132	3.81	55.33	
3.313	1.008	6.750	3.39	55.57	
9.448	Rhodan titration 0.590 c.c.				11.27
Cal. for $C_{15}H_{11}O_6Cl$			3.41	55.80	11.00

Properties of cyanidin chloride:

In amyl alcohol solution the colour is red violet which turns violet by aqueous sodium acetate and blue by ferric chloride. In alcoholic ferric chloride solution the colour is blue and changes into greenish yellow when it is diluted.

Oxidation test by aqueous ferric chloride was carried out in the same way as with hiviscin chloride. After three minutes fading of the colour occurred, and ten minutes later the colour was nearly oxidized and after in thirty minutes it was almost decolourized, The colour reaction in buffered solution showed the same colouration as is given by Robertson and Robinson[7] in the case of cyanidin chloride.

Analysis of carbohydrate:

The filtrate from the hydrolysis of hiviscin chloride wrs neutralized with sodium carbonate and was freed from the remaining colouring matter with amyl alcohol. The aqueous solution was then concentrated, added with alcohol to precipitate mineral matter, the alcoholic solution was evaporated, decolourized by animal charcoal and filled up 20 c.c. The sugar content was estimated by volumetric method.

I. 0.1066 g. anhydrous hiviscin chloride gave 0.042 g. xylose or 0.0392 g. arabinose.

II. 0.1430 g. anhydrous hiviscin chloride gave 0.043 g. xylose or 0.041 g. arabinose.

Since, in the cause of hydrolysis of hiviscin, evolution of furfural was observed, its content was estimated as phloroglucid. 23.307 mg. hiviscin chloride gave 4.974 mg. furfural phloroglucid corresponding to 7.746 mg. pentose (33.23 %) where $C_{20}H_{16}O_{10}Cl$ required 33.24% pentose. The maximum absorption of the above phloroglucid lies on 580 $\mu\mu$. This excludes the existence of methyl furfural phloroglucid, whose absorptions lie below 480.

The colour reaction of the carbohydrate:

(1) Naphthoresorcin test: green fluorescence, absorption on 520 $\mu\mu$, the colour was insoluble in ether. (2) Phloroglucin test: red. (3) Bial's test: positive (green). (4) Thomas' reaction: positive. (5) Pinoffs' test: positive, after a minute violet red.

(7) loc. cit., 37.

Derivatives:

Phenyl osazone was prepared in ordinary way. Recrystallized from 10 % alcoholic solution, m p 192° (yellow needle), *p*-brom-*o*-phenylosazone was also prepared. To 0.3 g. *p*-brom-*o*-phenylhydrazine in 3 c.c. hot water was added 0.1 g. sugar dissolved in 0.5 c.c. water and 1 c.c. of 50% aqueous acetic acid, and the mixture was heated on water-bath for 10 minutes. On cooling, the crystals separated out which recrystallized from 50% alcoholic solution form yellow needles of m p 162°.

The aqueous sugar solution is dextrorotatory:

These results indicate that the carbohydrate is a pentose and attached to cyanidin in molecular proportion, but further investigation is required in these respects.

The Catechin in the Fruit of *Areca catechu* L.

By

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(Received August 16, 1932.)

According to A. G. Perkin[1] areca catechin seems to resemble acacia catechin, but its pure isolation has not yet been succeeded. The fresh pulp of the fruit is colourless and soluble in water, but when it is dried in air it becomes insoluble brown matter, having an appearance of a phlobaphene. The fresh pulp must, therefore, be taken for a starting material.

The attempt of the isolation of catechin from the fresh fruit was successful. The crystal we had obtained was colourless needles m p 96° (the anhydrous form m p 175°). The analytical results agreed with $C_{15}H_{14}O_6$, and its optical properties and its penta-acetyl derivative $[C_{15}H_9O(OCOCH_3)_5]$ identity with *d*-catechin which we had isolated from gambier catechu[2][3].

Experimental.

The mashed pulp from 800 g. fresh fruit was extracted twice with each

(1) A. G. Perkin: The Natural Organic Colouring Matters, 463.
(2) A. G. Perkin and E. Yoshitake: J. Chem. Soc., **81** (1902), 1160.
(3) Freudenberg and Purrmann: Ann. Chem., **437** (1924), 274

1 L. alcohol (94%) for two hours at 80°. The united filtrate was evaporated in vacuum in the atmosphere of carbon dioxide, and the catechin was precipitated as lead salt, the latter having decomposed by sulphuretted hydrogen. The filtrate from lead sulphide was concentrated in vacuum to a syrup, which was then extracted with ether using a separating funnel. The ethereal solution was then concentrated and extracted with a little water. When this water solution was cooled, needle crystals separated, melting at 96°. The crystals contain 4 mol crystalline water to be dehydrated in vacuum at 100°. The anhydrous substance melted at 175°. Yield was 3 g.

With ferric chloride it gives a green colour, with bromine water a yellowish and with lime water a brownish red precipitate. It is precipitated when kept in contact with air, and other properties showed perfectly that it is identical with *d*-catechin from gambier.

Crystalline water:

1.0036 g. air-dried material lost at 100° in vacuum 0.2060 g.

in weight	H_2O	20.52%
Cal. $C_{15}H_{14}O_6 \cdot 4H_2O$	H_2O	19.89%

Analytical results:

Substance (mg.)	H_2O (mg.)	CO_2 (mg.)	H%	C%
5.219	2.014	11.887	4.32	62.13
2.982	1.078	6.836	4.42	62.53
$C_{15}H_{14}O_6$ requires			4.82	62.07

The rotation of *d*-catechin from *areca catechu*:

0.3125 g. was dissolved in 25 c.c. water containing a little aceton.

$$[\alpha]_D^{19} = (+ 0.20° \times 25) : (1 \times 1.25) = +16.0°$$

The absorption bands of spectrum were just the same as that of *d*-catechin from gambier.

Acetyl derivative:

0.5 g. dried material was dissolved in 7 c.c. well cooled pyridine, acetylated by 7 c.c. of acetylchloride. The mixture was then poured into ice-water and the precipitate was crystallized from alcohol acetone mixture; colourless needles, m p 128°, yield 0.6 g.

The analytical results:

The material dried at 100° in vacuum and analyzed.

Substance (mg.)	H_2O (mg.)	CO_2 (mg.)	H%	C%
5.458	2.357	12.001	4.79	59.96
3.119	1.349	6.858	4.80	59.96
$C_{15}H_9O(OCOCH_3)_5$ requires			4.80	60.00

The rotation of penta-acetyl *d*-catechin from *areca catechu*:
0.4446 g. dried substance was dissolved in 25 c.c. acetylene tetrachloride.
$[\alpha]_D^{19} = (+ 0.74° \times 25) : (1 \times 1.7784) = + 41.6°$.

On the Chemical Composition, especially Organic Bases of "Di-Saké".

By

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(Received September 12, 1932.)

The "di-saké" or "di-shu" is a kind of "saké", which is alcoholic beverage peculiar to the Japanese; and brewed in only two prefectures—Kagoshima and Miyazaki—in Japan. The annual production of "di-saké" in these districts amounts to about 3,400~3,600 *koku* or 6,200~6,500 hectolitres in round number. Its raw-materials, the same with "saké": that is (1) steamed rice, (2) "koji", the fungoidal mass of *Aspergillus Oryzae* grown on steamed rice, and (3) Water; but this steamed rice as raw-material of "di-saké" was made by the very roughly polished rice than that of the "saké", and then the method of brewing differ from "saké".

In the case of brewing of "di-saké", using in comparatively small amount of water and large amount of aged "koji", the saccharification of mash is promoted and the fermentation controlled; and to neutralize the acids in fermented mash, wood ash is added into the mash before the press; and then new "di-saké" obtained by the press is not pasteurized.

The "di-saké" is a sweet liquor of a yellow or yellowish brown colour and of the consistency more viscous than that of ordinal "saké"; and its use is not only for drinking, but for cooking instead of "mirin".

Experimental Results.

The "di-saké" experimented with was brewed at Kagoshima City; and the analytical results of the sample are shown in the following table;

	g. in 100 c.c.		g. in 100 g.
Crude protein	2.413		2.259
Protein	0.056		0.052
Extract	20.224		18.934
Reducing Sugar (as glucose)	12.880		12.059
Dextrin	1.652		1.547
Ash	0.260		0.243
Specific gravity		1.0681	
Alcohol	12.89		
" vol. %		16.24	
PH		6.823	

The various forms of nitrogen in the sample were also determined:

	g. in 100 c.c.	Ratio (Total N as 100)
Total N	0.386	100.0
Protein N	0.009	2.3
Non-protein N	0.377	97.7
In which Ammonia N	0.029	7.5
Organic base N	0.074	19.2
Other N	0.274	71.0

Isolation and Identification of Organic Bases.

For the isolation of the organic bases 10 litres of the "di-saké" were evaporated under reduced pressure to about 4 litres. After this operation the protein substance and other impurities were removed by neutral- and basic-lead acetate, and excess of the lead by H_2SO_4, and then the organic bases were precipitated by phosphotungstic acid. And according to the general method the precipitate formed by phosphotungstic acid was fractionated into three fractions, and researches were made about the organic bases in each fraction.

(1) Purine Base-Fraction ($AgNO_3$-precipitate):–

The yield of the base from this fraction was 0.20 g. as hydrochloride. Its picrate formed yellow prisms, changed to black colour at about 210°C (uncorr.). On analysing it as its chloroplatinate, following result was obtained:

0.0752 g. Subst. 0.0217 g. Pt. 28.86% Pt.
Calc. for Hypoxanthine-chloroplatinate $[(C_5H_4N_4O \cdot HCl)_2PtCl_4]$ 28.62% Pt.

The chloroaurate of the base formed yellow prisms, decomposed at 254° C (uncorr.), and the result of the analysis was as follows:

0.1647 g. Subst. 0.0682 g. Au. 41.41% Au
Calc. for Hypoxanthine-chloroaurat ($C_5H_4N_4O \cdot HCl \cdot AuCl_3$) 41.42% "

These results in all respects agree precisely with hypoxanthine derivatives.

(2) Arginine-Fraction ($AgNO_3$ & $Ba(OH)_2$-precipitate) :-

The yield of the base from this fraction was 4.60 g. as nitrate. The nitrate forms chalky substance, and the result of the analysis by Nitron method was as follows:

0.2049 g. Subst. 0.3193 g. $C_{20}H_{16}N_4 \cdot HNO_3$
0.05364 g. HNO_3 26.18% HNO_3
Calc. for Arginine-nitrate ($C_6H_{14}N_4O_2 \cdot HNO_3$) 26.55% HNO_3

The coppernitrate formed deep blue needles, melted at 112°C (uncorr.) and decomposed at 230°C (uncorr.).

0.1226 g. Subst. 0.0179 g. CuO 0.0143 g. Cu 11.67% Cu
Calc. for Arginine-copper-nitrate [$(C_6H_{14}N_4O_2) \cdot Cu(NO_3)_2$] 11.86% Cu

According to the above results this base seems to coincide with arginine.

(3) Lysine-Fraction (Filtrate from $AgNO_3$ & $Ba(OH)_2$-precipitate) :-

The hydrochloride, which was freed from water obtained by this fraction, was treated with cold absolute alcohol and separated into two portions.

(a) Insoluble portion by cold absolute alcohol :-

Yield ; 0.50 g. This chloride was identified as KCl.

(b) Dissolved portion by cold absolute alcohol :-

Saturated alcoholic solution of $HgCl_2$ was added to this portion.

The hydrochloride of the base obtained from the $HgCl_2$-precipitate, forms colorless, very hygroscopic, large prisms and gives the alloxan reaction. The chloroaurate prepared from the hydrochloride forms yellow mossy crystals, and is sparingly soluble in water ; the melting point was determined as 256—257°C(uncorr.) ; and the analysis gave the following results :

0.1541 g. Subst. 0.0689 g. Au 44.71% Au
0.2605 g. " 0.1157 g. " 44.41% "
Calc. for cholinechloroaurate ($C_5H_{14}NOCl \cdot AuCl_3$) 44.49% "

The chloroplatinate formed orange yellow prisms, easily soluble in water, melted at 236—237°C (uncorr.) with decomposition, and gave the following analysis :

0.2345 g. Subst. 0.0734 g. Pt 31.30% Pt
0.3581 g. " 0.1118 g. " 31.22% "
Calc. for cholinechloroplatinate [$(C_5H_{14}NOCl)_2PtCl_4$] 31.64% Pt

The above results agree fairly with choline derivatives ; the yield of the base was 6.75 g. as chloroaurate.

Summary.

The chemical composition of the "Di-saké" compared with that of the ordinal "saké" is summarized as follows :

(1) In the above experimental results the nitrogenous compounds isolated from 10 litres of "di-saké", are hypoxanthine-hydrochloride (0.20 g.), arginine nitrate (4.60 g.), cholinechloroaurate (6.75 g.), and ammonia (3.52 g.), while the isolated bases from 25 litres of "saké" by Dr. K. Kurono, are cholinepicrate (3.8 g.), histaminepicrate (0.15 g.), lysinepicrate (0.52 g.) and ammonia (1.3 g.). It is the most great difference that the "di-saké" contains large amount of arginine, which could not be isolated from the "saké".

(2) As regards the general composition, 1 fined great difference between the "di-saké" and the "saké": in the former, the quantity of reducing sugar (chiefly glucose) and other extractive substances are considerably much more than those of the latter.

(3) In the "di-saké", the amount of non-protein substances is much more than those of the "saké".

(4) The reaction of "di-saké" is almost neutral, but in the "saké" remarkably acidic.

Researches on the Electrolytic Reduction Potentials of Organic Compounds, Part XVI.

Reduction potential of *p*-aminoazobenzene.

By

Masuzo SHIKATA and Isamu TACHI.

(Received September 19, 1932.)

Summary.

The results of the investigation on the electrolytic reduction potential of *p*-aminoazobenzene with the polarograph and dropping mercury cathode at 25°C were as follows.

(1) Reduction potential of *p*-aminoazobenzene was more positive in lower concentration than that in higher concentration in every PH solution.

(2) Owing to the weak basic property of *p*-aminoazobenzene, there were two reduction potentials which were due to the dissociated and undissociated forms in some proper acidic solutions. In high acidic solution, *p*-aminoazobenzene changed to quinoid form which was demonstrated by spectrographic study. The reduction potential of quinoid form was more positive than that of azoid form and further, that of dissociated form more posi-

tive than that of undissociated form.

(3) The reduction potential of *p*-aminoazobenzene which substituted NH_2 group in azobenzene was more negative than that of the latter. This is expected from our negativity rule of electrolytic reduction.

(4) The solubility of *p*-aminoazobenzene in water at 25°C was found to be $2.815 \cdot 10^{-4}$ g. mol per litre calculated by the result of polarographic measurement.

Researches on the Electrolytic Reduction Potentials of Organic Compounds, Part XVII.

Reduction potential of dimethylaminoazobenzene.

By

Isamu TACHI.

(Received Seppember 19, 1932.)

Summary.

The results of the investigation on the electrolytic reduction potential of dimethylaminoazobenzene with the polarograph and dropping cathode were as follows:

(1) The reduction potential of dimethylaminoazobenzene was more negative in high concentration than that of in low concentration, except in the case of solution which PH was lower than 2.2.

(2) In acidic solution, the reduction of dimethylaminoazobenzene showed the reduction potentials of quinoid, dissociated and undissociated forms of azoid form as same as in the case of *p*-aminoazobenzene.

(3) The maximum currents of the polarograms of dimethylaminoazobenzene in acidic solutions were increased with elevation of concentration of ethanol. From these facts, we assume that dimethylaminoazobenzene becomes easily adsorbable on the mercury cathode in acidic solutions.

(4) The mutual relation among the reduction potentials of azobenzene, *p*-aminoazobenzene and dimethylaminoazobenzene was found to follow our negativity rule of electrolytic reduction, that is, reduction potential of azobenzene was most positive and that of dimethylaminoazobenzene most negative among them.

Ueber die Verdauungsenzyme der Seidenraupen (*Bombyx Mori*, L.)

Von

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(Eingegangen am Oktober 9, 1932.)

I. Einleitung.

Als Verdauungsenzyme der Seidenraupen haben mehrere Forscher Amylase, eine trypsinähnliche Protease und Lipase in den Säften der Digestionskanale, aber Invertase als Endoenzym in den Wänden derselben festgestellt.

Zur rationellen Züchtung der Seidenraupen, ist es nötig, die Natur dieser Enzyme gründlich zu berücksichtigen. Zur Untersuchungen der Erblichkeit der chemischen Eigentümlichkeiten in den Lebewesen, hat der Verfasser die Wirkungskräfte dieser Enzyme im Körper der Raupen beobachtet, und zwar zuerst inbezug auf die Invertase, deren Enzymnatur nicht genügend bekannt ist, er erforschte den Einfluss der Wasserstoffionenkonzentration und Temperatur auf die Enzymwirkung, und auch bezüglich der Amylase und Protease bestimmte ihre Wirkungskraft in einigen Originalrassen der Seidenraupen.

II. Einfluss der PH und Temperatur auf die Invertasewirkung.

1) Darstellung der Enzymlösung:- Die aus den Seidenraupen herausgenommene Wände der Digestionskanale wurden zur Entfernung des Saftes, des Blutes und anderer Substanzen, zunächst mit 0.85 proz. Kochsalzlösung und dann mit Wasser genügend gewaschen. Diese Wände wurden in einer Reibschale mit feinkörnigem Quarzsand gut verrieben; das Gemenge mit wasserhaltigem Glycerin verrührt, nach eintägigem Stehen bei Zimmertemperatur zentrifugiert und die überstehende Flüssigkeit filtriert. Die so erhaltene Enzymlösung ist durchsichtig.

2) Bestimmungsmethode der Enzymwirkung:- Ich verwandte Sörensensche Zitrat-, Phosphat- und Glykokoll-gemische als Pufferlösungen. Die Saccharoselösungen, unter verschiedenen Pufferzusätzen, wurden mit Enzymlösungen gemischt, nach verschiedener Versuchsdauer wurden 2 *N* Sodalösungen zugesetzt und die Drehungsabnahme gemessen.

3) Einfluss der PH:- Ich will im folgenden ein Beispiel der Experimente kurz beschreiben. 4 ccm. 30% iger Saccharoselösung wurden mit 4 ccm. Enzymlösung, 8 ccm. Pufferlösung und Toluol versetzt. Nach 18 Stun-

den bei 37°C wurden 10 ccm. dieser Flüssigkeit in 2 ccm. 2 *N* Sodalösung zugesetzt und der Drehungsgrad beobachtet.

PH	4.64	5.01	5.75	6.23	6.59	7.06
Drehungsabnahme (°)	0.06	0.35	0.99	0.80	0.80	1.04
Saccharosespaltung (%)	1.27	7.42	20.97	19.07	19.07	22.03
PH	7.53	7.78	8.34	8.68	9.45	9.82
Drehungsabnahme (°)	0.84	0.85	0.94	1.10	1.12	0.78
Saccharosespaltung (%)	17.80	18.01	19.92	23.31	23.73	16.53

Die Ergebnisse des oben erwähnten und anderer Versuche, welche im Jour. Agr. Chem. Soc. Japan (Japanisch) dargestellt worden sind, weisen darauf hin, dass die saccharosespaltende Kraft dieses Enzyms sehr schwach ist, unter PH = 4.0 hat es fast keine Wirkung, aber bei alkalischer Einwirkung ist sie etwas höher und zwischen PH = 5.5 und 10.0 besteht nahezu die gleiche Wirkungskraft und bei PH = 9.5 liegt das Wirksamkeitsoptimum.

Diese Resultate interessieren im Vergleich zu der Tatsache dass, die Wasserstoffionenkonzentration des Digestionssaftes und des Blutes der Seidenraupen nahe PH = 6.5 und PH = 10.0 liegt; bei PH 9.0~10.8 liegt die optimale Wirkungen der Amylase und Protease des Digestionssaftes.

4) Einfluss der Temperatur:- Zu 5 ccm. 30%iger Saccharoselösung wurden 15 ccm. Glykokoll-Puffergemisch von PH = 9.5 addiert. Diese Flüssigkeiten und 10 ccm. Enzymlösungen wurden auf 20 Min. bei verschiedenen Temperaturen gehalten, gemischt und nach 20 Min. bezw. 40 Min. je 10 ccm. dieser Lösungen mit 2 ccm. 2 *N* Sodalösung versetzt. Die unter diesen gleichartigen Bedingungen durchgeführten Untersuchungen zeitigten die nachstehenden Ergebnisse:

Nach 40 Min.

Temperatur (°C)	10.0	25.0	31.0	37.0	45.0	55.6	70.0
Drehungsabnahme (°)	0.32	0.36	0.39	0.41	0.45	0.25	0.15
Saccharosespaltung (%)	9.30	10.47	11.34	11.92	13.08	7.56	4.36

Die Invertasewirkung ist also selbst bei 10°C beträchtlich, wird mit steigender Temperatur stärker und erreicht das Optimum bei 45°C, wird dann aber nach 40 Min. bei 70°C zerstört.

III. Beziehungen zwischen den Rassen der Seidenraupen und den Wirkungskräften der Amylase und Protease der Digestionssäfte.

1) Rasse und Züchtung:- Ich Wählte Japan-Gynryu, Japan-110, China-7-B, China-7-D, Europa-7-A und Europa-7-C als Originalrassen; China ×

Europa-E und China × Europa-F als Bastardrassen aus. Diese 8 Rassen wurden unter gleichen Bedingungen zu derselben Jareszeit aufgezogen.

2) Gewinnung des Digestionssaftes:- Wenn die Seidenraupen Chloroform riechen, brechen sie die Verdauungssäfte aus. Diese Säfte wurden in Gefässe mit Toluol gesammelt und sofort gebraucht.

3) Bestimmungsmethode der Enzymwirkung:-

a) Dextrinbildung aus Stärke:- Die 1%igen Stärkelösungen wurden mit den gleichen Volumina M/10 Glykokoll-Puffergemisch von P_H = 9.08 vermischt. Als Enzymlösung wurde stets der mit 0.85%iger Kochsalzlösung verdoppelte Verdauungssaft benutzt und im wesentlichen nach dem Verfahren von Wolgemuth die Enzymwirkung bestimmt.

b) Verzuckerung der Stärke:- 15 ccm. 5%iger Stärkelösung wurden mit 15 ccm. M/10 Glykokoll-Puffergemisch von P_H = 8.67 vermengt, diese Lösung, nach 20 Min. bei 30°C, durch den mit 0.85%iger Kochsalzlösung verdoppelten Digestionssaft auf eine Stunde bei 30°C stehen gelassen und der entstandene Zucker mittels der Methode von Bertrand bestimmt.

c) Eiweissspaltung:- Die in M/10 Glykokoll-Pufferlösung von P_H = 10.74 gelöste 0.25%ige Kaseinlösung wurde durch Enzymlösung, welche mit 0.85%iger Chlornatriumlösung hundertfältig verdünnt wurde, eine Stunde bei 30°C stehen gelassen, das ungespaltene Substrat mit 1.5 %iger Trichloressigsäure gefällt und nephelometrisch bestimmt.

4) Ergebnisse der Versuche:- Verf. gibt einige seiner Versuchsresultate in der Tabelle wieder. Die Zahlen in dieser Tabelle erweisen: Dextrinbildung —— ccm. 1 proz. Stärkelösung, welche durch 1 ccm. Digestionssaft in einer Stunde bei 30°C und P_H = 9.08 in Dextrin gespalten wurden; Verzuckerung —— ccm. 0.5 %iger Kaliumpermanganatlösung, welche dem durch 1 ccm. Digestionssaft während einer Stunde bei 30°C und P_H = 8.67 entstandenen, aus Stärke invertierten Zucker entsprechen; Eiweissspaltung —— mg. Kasein, welche durch 1 ccm. des mit 0.85 %iger Kochsalzlösung hundertfältig verdünnten Digestionssaftes während einer Stunde bei 30°C und P_H = 10.74 gespalten wurden.

	Japan-Ginryu	Japan-110	China-7-B	China-7-D	Europa-7-A	Europa-7-C	China-Europa-E	China-Europa-F
Dextrinbildung	7.5	10.0	10.0	30.0	0.0	0.0	10.0	7.5
Verzuckerung	5.58	8.68	8.68	18.60	1.86	0.62	4.96	4.96
Eiweissspaltung	33.34	33.50	34.82	31.90	33.34	32.41	32.09	32.81

Die stärkespaltende Kraft, sowohl die Dextrinbildung, als auch die Verzuckerung, ist am stärkesten bei China-7-D; danach bei China-7-B und Japan-110; und darauffolgen Japan-Ginryu, China × Europa-E und China × Europa-

F; bei Europa-7-A und Europa-7-C aber ist sie sehr schwach. Die anderen experimentelle Data, die in der Originalarbeit beschrieben wurden, zeigen dass diese Kraft bei gleichem Alter von Tag zu Tag stärker wird. In der Eiweissspaltbarkeit des Digestionssaftes, unter den oben erwähnten experimentellen Bedingungen, besteht ein Unterschied in den Rassen der Seidenraupen nicht.

Zusammenfassung.

1) Die Invertase, welche in den Wänden des Digestionskanals der Seidenraupen vorkommt, wirkt zwischen $P_H = 5.5$ und $= 10.0$ ungefähr gleich stark und zwar liegt das Optimum etwa bei $P_H = 9.5$

2) Die saccharosespaltende Kraft dieser Invertases wird mit steigender Temperatur stärker; das Maximum liegt bei 45°C. Bei 70°C wird sie innerhalb 1 Stunde zerstört.

3) Unter 8 Rassen der Seidenraupen, besteht die folgende Reihenfolge für die Wirksamkeit der in den Digestionssäften vorkommenden Amylase gefunden: China-7-D>China-7-B>Japan-110>Japan-Ginryu, China × Europa-E, China × Europa-F>Europa-7-A, Europa-7-C.

4) Es besteht kein Unterschied in der Kaseinspaltbarkeit der Verdauungssäfte der verschiedenen Rassen.

A Study on the Effects of Fatty Acid on Nutrition

By

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Until recently, it has been considered that the essential function of fats in the body is to act merely as fuel to the tissues and that when the supply of fat-soluble and other vitamins are sufficient fats can be omitted from the dietary[1][2][3].

However, since in 1928, Evans and Burr[4] proved definite subnormal

(1) Hinhede: Chem. Zentr., **2** (1918), 745.
(2) T. B. Osborne and L. B. Mendel: J. Biol. Chem., **45** (1920—1921), 145.
(3) J. C. Drummond and K. H. Coward: Lancet, **2** (1921), 698.
(4) H. M. Evans and G. O. Burr: Proc. Soc. Exp. Biol. and Med., **25** (1928), 390.

growth and irregular ovulation on diet which was complete in every respect except for fats, a considerable emphasis has been put on the fat requirement of the animal. The next year, Burr and Berr[5] noted a new dietary deficiency in fat-free diet, by which rats developed a characteristic symptom, the so-called scaly tail condition. About the same time, McAmis, Anderson, and Mendel[6] reported subnormal weight of animals on a low fat diet, thus supporting the view that fats are beneficial to the rats. Furthermore, Burr and Burr[7] indicated that linoleic acid was highly important and the rats receiving the acid lost the scales and dandruff from their feet and back and were cured. This investigation called the author's attention to determine what kinds of fatty acids and their glycerides are essential and what *rôle* they play in nutrition.

The present paper is concerned with some dietary deficiency developed in the rigidly fat-free diet and with the *rlôe* of unsaturated fatty acids.

Preparation of Basal Diet and of Animals.

Casein:- 110 g. of high grade casein were washed with distilled water twice daily for 2 days. After draining the washed water, the casein was transferred into 1100 c.c. of 0.09 normal NaOH with a little amount of toluene and shaken for about 20 hours. The alkaline solution was then treated with a 1 : 1 mixture of normal hydrochloric acid and acetic acid under violent stirring. The precipitated casein was washed with distilled water until the chloride test was almost negative, and the water was pressed out by suction. The casein was now put into 95 per cent alcohol to remove the moisture. After pressing out the alcohol as completely as possible, the casein was spread on the filter paper to dry at ordinary temperature; the dry powdered casein was extracted with ether for 7 days. The prolonged extraction was very important in producing a casein free from fat.

Starch:- Potato-starch (Japanese pharmacopoeia) was boiled with 95 per cent alcohol containing hydrogen chloride gas according to the direction given by Taylor and Nelson[8]. The purified starch obtained in this way was extracted with ether for 7 days. Later, the procedure was modified by mere extraction with 95 per cent alcohol, as there were no different effects upon the rats. Taylor and Nelson have shown that the major parts of the fatty substance present in starch cannot be removed by solvent without previous hydrolysis. They have also indicated that in the case of potato-starch there is only a trace of the "fat by hydrolysis", which is almost negligible. Moreover, recent investigation of Evans and Lepkovsky[9] has proved that potato-

(5) G. O. Burr and M. M. Burr: J. Biol. Chem., **82** (1929), 345.
(6) A. J. McAmis, W. E. Anderson and L. B. Mendel: J. Biol. Chem., **82** (1929), 247.
(7) G. O. Burr and M. M. Burr: J. Biol. Chem., **86** (1930), 587.
(8) T. C. Taylor and J. M. Nelson: J. Am. Chem. Soc., **42** (1920), 1726.
(9) H. M. Evans and S. Lepkovsky: J. Biol. Chem., **96** (1932)' 143.

starch was ineffective as a cure of the disease that resulted from fat-free diet. Yeast extract:- 400 g. of dry powdered yeast (baker's yeast prepared by Oriental Yeast Company in Tokyo) were added to 1600 c.c. of 50 per cent ethylalcohol and stirred continuously for 1/2 hour at room temperature; then the mixture was filtered through muslin in a fruit-press or a Buchner funnel. The residue was treated in like manner with 65 per cent alcohol and filtered as before. The residue, this time, was transferred into 75 per cent boiling alcohol of the same volume as above, the boiling was continued for one hour, let settled for some hours, and then filtered. The treatment was repeated once more, but with 85 per cent alcohol.

All the filtrates were combined and evaporated under reduced pressure. The concentrated extract was then slightly acidified with hydrochloric acid and shaken with ether to remove fat completely; the ether was evaporated off. The solution was diluted with distilled water to a volume of 400 c.c. and preserved in the ice-box. This extract should have contained all of the water-alcohol-soluble vitamin B complex of yeast. Each c.c. was equivalent to 1 g of the original yeast.

Basal diet:- The diet consisted of 21% purified casein, 75% purified starch and 4% McCollum salt mixture, supplemented with 5 c.c. of the yeast extract for every 100 g of the diet. As vitamin A and D sources, one drop of 1% biosterol* free from fat and of 0.01% irradiated ergosterol† were given per rat per day respectively, both dissolved in liquid paraffin. Later, 0.1% ergosterol solution was used since 0.01% solution might be insufficient to prevent rickets. Iodine water was furnished once a week.

Animals:- In all the experiments male albino rats of 35—40 g. of weight were used, and 2 or 3 of them were kept in a cage with the raised bottom of coarse wire-screen to prevent accessibility to feces. The solid fatty acids were simply mixed with the basal diet while the liquid fatty acids were given by means of a dropping pipette. Positive results were marked by the recovery of symptoms and renewed growth. In our laboratory the diet is usually cooked in a semisolid state to prevent spilling.

Fatty Acids Used and Their Preparation.

The fatty acids** used in this experiment are listed as Table I[10].

Stearic acid was recrystallized from alcohol.

Clupanodonic acid was prepared from methyl ester of the acid according to the direction of Sahashi: 40 g of the methyl ester were dissolved in 400

*† I wish to thank Dr. M. Sumi and Mr. S. Hamano for supplying the irradiated ergosterol and biosterol.

** I desire to thank Dr. Y. Sahashi for generously supplying the pure fatty acids.

(10) Y. Sahashi: "Riken Iho", 11 (1932), 1075.

cc of 5% alcoholic NaOH and left overnight at room temperature, then the solution was diluted with distilled water twice the volume. The solution

Table I.

	b p/m p	d	n_D	Acid value	Iodine value
Oleic acid $C_{18}H_{34}O_2$	b p=181—2°C (0.18—0.2 mm.)	$d_4^{18}=0.9106$	$n_D^{20}=1.4390$	197.6 Calc. 198.6	93.7 Calc. 90.1
Elaidic acid $C_{18}H_{34}O_2$	b p=204—5°C (0.4—0.5 mm.) m p=43—4°C	——	——	201.0 Calc. 198.6	88.9 Calc. 90.1
Linoleic acid $C_{18}H_{32}O_2$	b p=193—4°C (0.1 mm.)	$d_4^{18}=0.9248$	$n_D^{20}=1.4685$	201.5 Calc. 200.0	181.5 Calc. 181.4
Linoleic acid $C_{18}H_{30}O_2$	b p=200—1°C (0.4—0.5 mm.)	$d_4^{18}=0.9239$	$n_D^{20}=1.4778$	200.8 Calc. 200.6	265.8 Calc. 273.9
Clupanodonic acid (Japanese Iwashi san)*					
Stearic acid (Kahlbaum) $C_{18}H_{36}O_3$	69—70°C				

was now extracted twice with petroleum ether below b p 50°C. After separating the water layer from the petroleum ether the water solution was acidified with HCl, and again extracted with the petroleum ether. After dehydrating with anhydrous Na_2SO_4, the petroleum ether solution was evaporated as completely as possible in a high vacuum in CO_2 atmosphere.

The methyl ester was assigned by Sahashi[10] as in the following table; its chemical structure is under investigation.

Table II.

	b p/m p	d	n_D	Acid value	Iodine value
"Iwashi san" methyl ester	b p=174—5°C (0.018—0.02 mm.)	$d_4^{20}=0.9290$	$n_D^{20}=1.4868$	174.2 (titrated as acid)	340.0 (Rosemund) 338.0 (Wij's method)

His remarks on the preparation of the fatty acids mentioned above are summarized as follows:

Oleic acid was prepared by redistilling, using mercury pump, a special preparation which contained no linoleic acid made by E. Merck Company: Eleidic acid was made from Merck oleic acid of German pharmacopoeia by

* M. Tsujimoto gave the formula $C_{18}H_{28}O_2$ for clupanodonic acid.

treating it with sodium nitrite and nitric acid (d=1.2), it was converted into Pb-salt and then was recrystallized from benzene. After separating as free acid and distilling in order to secure the removal of a trace of Pb, it was recrystallized from alcohol: Linoleic acid and linolenic acid were prepared from soy bean oil and linseed oil respectively according to the method given in "Riken Iho"[11]: By saponifying with alcoholic potassium hydroxide, brominating in ether solution, collecting the resulted tetrabromostearic acid (m p 114°C), reesterifying with methyl alcohol containing hydrogen chloride gas, and distilling in a high vacuum; obtaining the free acids by saponifying with alcoholic potassium hydroxide at ordinary temperature, and by distilling in a high vacuum in CO_2 atmosphere.

From the data of the analysis, it can be recognized that the fatty acids used in this work were 100 per cent pure.

Results.

The results obtained from feeding were divided into two parts according to the different periods of the experiment:

	A. First part (Chart I—II)	B. Second part (Chart III—VII)
Period	January—April	April—August

During the first period the animal room was maintained at a moderate temperature, average 70°F, and the animals attained growth regularly, whereas for some intervals of the second period, especially in the later stage, it was raised to a temperature as high as 85—95°F, and the growth of some animals was irregular and retarded even when administered with unsaturated fatty acids, and though its improvement was usually expected, sometimes death happened suddenly.

A. First Part.

a) The animal group fed on the basal diet and administered with 3 drops of linoleic acid daily (0.078 g. corresponding to about 0.5%) grew healthy; the hair was very fine and lustrous. It was especially noticeable that the eyes were extremely clear and bright (Chart I).

b) The group fed with oleic acid instead of linoleic acid showed the sign of losing hair around the nose and the mouth. First, swelling of the front paws was noticed, and later, slight "scaling", to be described below, appeared on the hind legs. But this symptom seemed to be specific since the growth was continuing at an approximately normal rate (Chart I).

(11) Y. Sahashi: Ibid, **10** (1931), 578.

c) The rats reared on the basal diet alone developed a sign of nutritional disorder between the 7th and the 8th week of the experiment. First, the loss of hair around the nose, the mouth; and the eyes took place, and there appeared a tendency to lose the hair on the chest and under arms, sometimes spreading to the back. The period at which baldness occurred, the regions of the body first affected, and the character of the loss of hair varied greatly among individuals, but the areas of baldness were usually symmetrical. The bald areas looked moist and inflamed. Later, there were swelling and hemorrhage of the nose and the mouth. In severe cases; the tip of the tongue and the lips underwent necrosis and dropped off in the manner of gangrene and sometimes inside of the mouth cavity became necrotic. Dermatitis appeared while these symptoms were developing and the exudate dried on the skin, where fine silky hair had matted, formed crusts resembling "scales". In all cases, the front paws were swollen and at times hemorrhage at the joints or necrosis at the end of the front paws appeared, and the hind paws became "scaly". The eyes were swollen and often closed by accumulation of secretion, but there was no change in the cornea as in the case of xerophthalmia due to the deficiency of vitamin A.

Blood urine or occult blood in the urine was not often observed in these rats, although Evans and Lepkovsky[9] stressed on hematurea as an important part of the symptoms.

The growth was retarded with the development of the symptoms so far described, then the decline of the body weight followed and the rats were observed to sit in a humped position. When this condition continued, the animals could not live much longer unless they were administered with either linoleic acid or linolenic acid. On healing, a soft fine coating of hair appeared on the denuded areas after a few days, and growth proceeded rapidly and the animal soon resumed a normal appearance.

On post-mortem examination there was no definite macroscopic change found in the internal organs except atrophy of lymphoid system and fatty degeneration of liver in some cases.

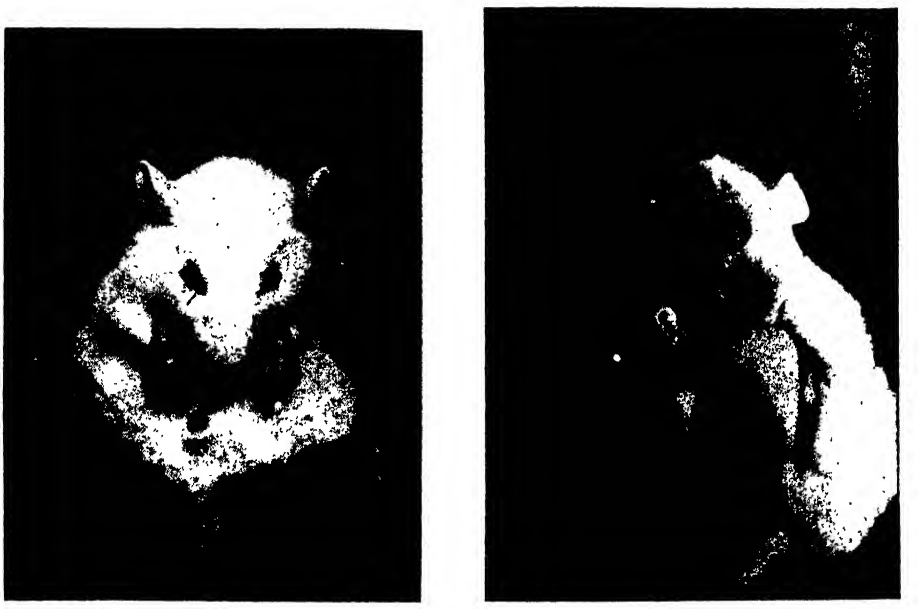
The symptoms developed on the fat-free diet seem to be a kind of dermatitis accompanying no suppuration and strikingly resemble pellagra recorded in the literatures and also a disease resulted from high white egg diet reported by Parsons[12] in some respects. Some examples are illustrated by the photographs.

Fig. 1 illustrates some of the symptoms produced on fat-free diet. The rat (a) died two days after the photograph had been taken, and the rat (b) died soon after the photograph had been taken. [Refer to (A) and (B) in

(12) H. T. Parsons: *J. Biol. Chem.*, **90** (1931), 351.

Chart II].

The rat (a) in Fig. 2 had the symptoms mentioned above, and was suffering from inflamation of chest, swelling of paws, "scaling" of hind legs and pus under arms. This rat lost its weight gradually, and then suddenly from



(a) (b)

Fig. 1.

116g to 94g within only two days. When such a rapid decrease occurred, death happened soon. By administering 2 drops of linoleic acid daily, the rat regained its weight, and in a few days pus under arms disappeared and a new skin was formed. Its weight increased to 150 g during the treatment with the acid for 20 days and the rat (b) recovered completely. (Refer to E in Chart II).

Fig. 3 shows the process of recovering of the "scaly" feet developed with fat-free diet. The rat had about the same symptom as the rat in Fig. 2 (a). As soon as 3 drops daily of linoleic acid were administered, the rat renewed its growth, "scaly" feet disappearing: a) before treatment, b) 11th day after starting daily administration of 3 drops of linoleic acid, c) 14th day

(a) 94 g.

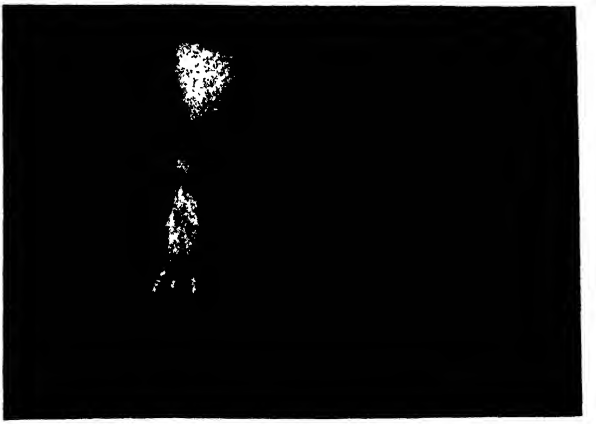
(b) 150 g.

Fig. 2.

(a)

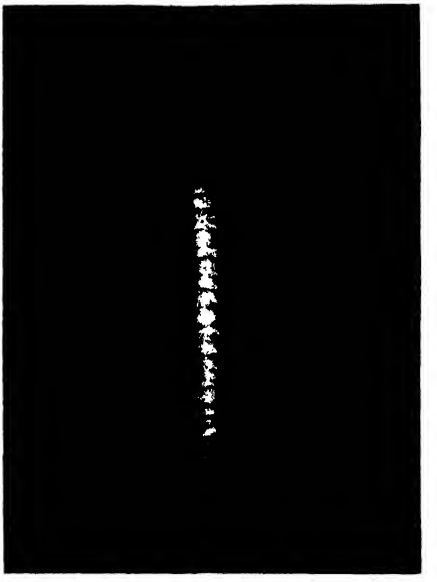
(b)

Fig. 3.



(c)

(d)



(e)

Fig. 3.

beef liver. The lesion, however, was cured by administering 2 drops of linoleic acid daily ; d) tail lesion and e) complete recovery of the symptom. after same treatment. (Refer to F in Chart III).

In the course of recovery, this rat has shown a tail lesion nearly identical with that on rich egg white diet described by Parsons[12], who claimed that neither 10 drops daily of linseed oil nor commercial lard were effective for cure, but recovered completely when administered with 20 per cent of dried

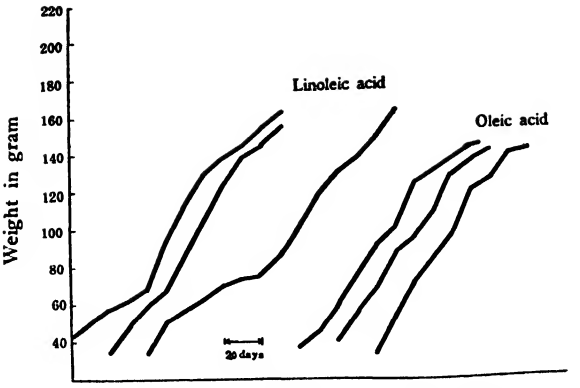


Chart I—Showing the effects on growth of administering 3 drops daily of linoleic acid and of oleic acid.

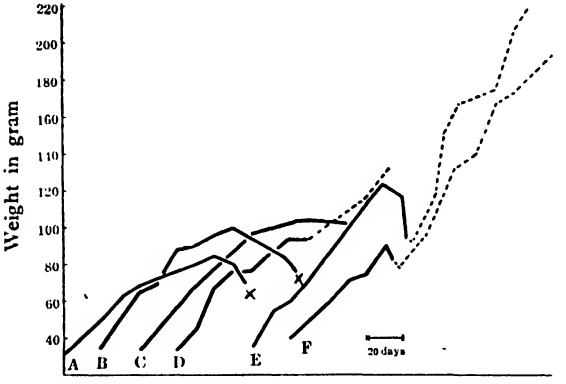


Chart II—Showing the effects on growth of basal diet alone, and the renewed growth and rapid recovery from the deficiency disease by the treatment with linoleic acid.
× indicates death.

B. Second Part.

Since linoleic acid had shown a remarkable effect in curing the fat deficiency, a study of the other acids containing 18 carbon atoms, saturated and unsaturated, was undertaken.

a) Linolenic acid:- One drop daily of this acid was used. When growth was somewhat retarded, as shown in Chart III *a*, the dose was increased to 2 drops, but no great improvement could be obtained. This retardation and irregularity, not found with linoleic acid, seemed to be rather

influenced by heat as seen in Chart III *a*.

As in Chart III *b*, it was proved that linolenic acid was almost identical with linoleic acid in curing rats suffering from deficiency of fat (Chart III *a* and *b*).

b) Clupanodonic acid:- One drop of the acid was used daily, but the feeding on this acid was found difficult on account of its characteristic fishy smell and its easy oxidation in the air: the rats ejected the acid; the acid changed to dry, varnish-like mass in the course of a few days unless special precautions were taken to prevent oxidation, and so on. One rat showed a very remarkable growth with nearly a normal appearance, while the other gained weight for a short interval after being fed on the dose; then lost appetite and began to decline and death was preceded by a rapid loss of weight. The bareness of the back, the chest and paws, and around the eyes and the mouth was noticed, but "scaly" feet never occurred on these rats. Diarrhea was the most noticeable feature,

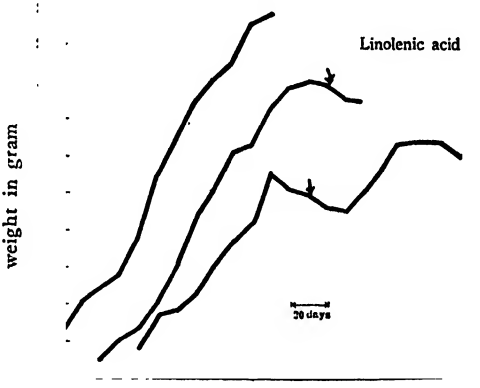


Chart III *a*.—Showing the effects on growth of administering one or two drops daily of linolenic acid. The arrow indicates increase of the acid from one drop to two.

An additional experiment was performed to see the effects of linoleic acid and linolenic acid on the sick animals produced on this dose. Two out of three sick animals were fed with 2 drops of linoleic acid and the third with the same amount of linolenic acid.

Unfortunately, neither of the acids showed any beneficial effects upon these rats and they soon died. This failure in curing might be due to the fact that emaciation and malnutrition advanced too far as they did not much take in the diet; thus this experiment was of little value (Chart IV).

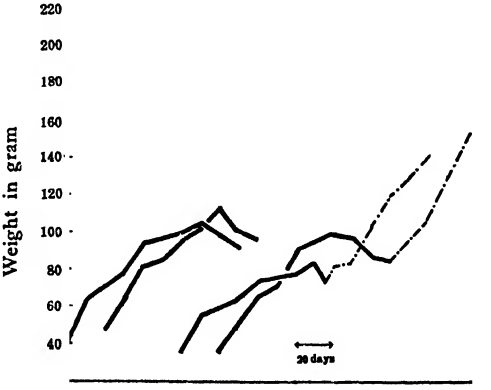


Chart III *b*.—Along with the renewed growth there is rapid recovery from the deficiency disease.

c) Linoleic acid with

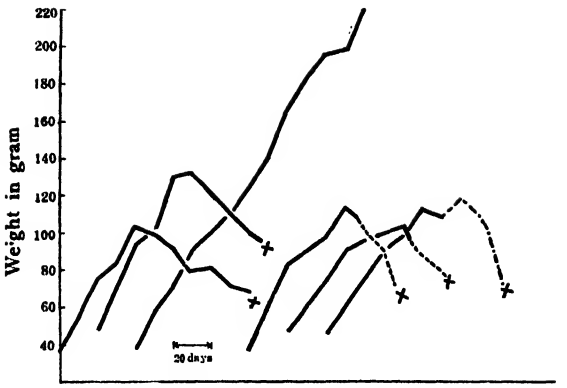


Chart IV.—Showing the effects on growth of administration daily with one drop of clupanodonic acid. × indicates death.

active oryzanin* instead of the yeast extract:– This was conducted with the object to determine whether linoleic acid could spare vitamin G as suggested by Hume and Smith[13]. Two drops of linoleic acid and one drop of 10% active oryzanin solution daily (each drop corresponding to 4 mg. of the original oryzanin) were used; later, the doses were increased to 3 drods of the acid and 2 drops of oryzanin solution. Unfortunately, however, though the rats grew for a short period on this diet, the growth became practically stationary, and the rats gradually lost weight and died except one rat. The symptoms as already mentioned on the fat-free diet were not remarkable but for a tendency to produce incrustation on the tail.

By replacing the oryzanin solution with the yeast extract, the rat soon gained weight and resumed a normal appearance. When it was changed again to the oryzanin solution, the rat began to lose weight (Chart V).

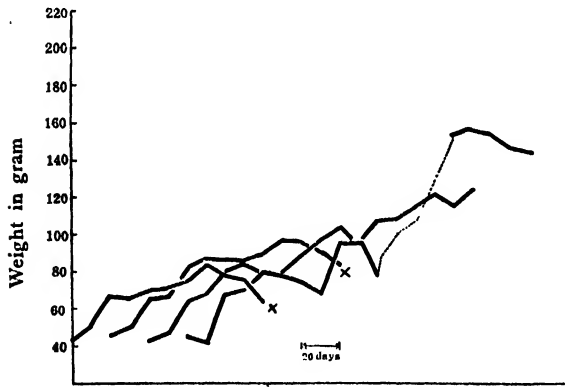


Chart V.—Showing the effects on growth of supplementing with oryzanin solution as vitamin B source instead of yeast extract, and the renewed growth by replacing oryzanin solution with yeast extract. × indicates death.

It seems, therefore, as though fat has no sparing action on vitamin G.

d) Elaidic acid:- 5 rats were reared on the diet containing 0.5 per cent elaidic acid. There were two distinct features appearing in this group: In one case the rats gained weight, while in the

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I am indebted to Dr. S. Odake for supplying the active oryzanin which cured severe antineuritic pigeons with 4 mg. daily.

The same yeast extract as used in the whole experiments was employed, and its curing power on pellagra rats has been measured previously.

(13) E. M. Hume and H. H. Smith: Biochem. J., 25 (1931), 292.

other they suddenly lost weight. Such a phenomenon also appeared in the experiments of Sahashi[9]. The reason for these two different features is not fully understood. In the former, if such is the case, the biological value of elaidic acid seems to be superior to that of oleic acid. None of the rats showed the "scaly" feet. In the latter, however, one rat died though administered with 2 drops of linolenic acid, while the other regained weight (Chart VI).

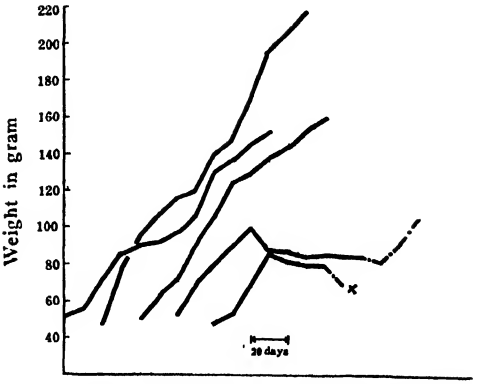

Chart VI.—Showing the effects on growth of adding 0.5 per cent of elaidic acid to the basal diet. × indicates death.

e) Stearic acid:— The diet containing 0.5 per cent recrystallized stearic acid was used in this experiment. The rats receiving the stearic acid diet exhibited a remarkable growth for some interval, and suddenly lost weight and died. Evans and Lepkovsky[9] stated that animals on the stearic acid diet were in poorer condition than those on fat-free diet, and this might be due to the fact that the acid was poorly absorbed (Chart VII).

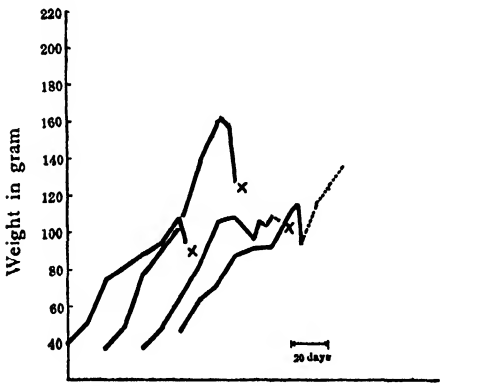

Chart VII.—Showing the effects on growth of adding 0.5 per cent of stearic acid to the basal diet. × indicates death.

Summary.

1. It is confirmed that both linoleic acid and linolenic acid are equally effective in curing a specific deficiency disease produced by rigidly fat-free diets.

2. Oleic acid and elaidic acid induce growth response of rats, but seem to be ineffective in curing sick rats.

3. The rats receiving clupanodonic acid suddenly lose body weight and are not cured with either linoleic acid or linolenic acid.

4. The poor results obtained with stearic acid may be attributed to imperfect absorption.

5. Vitamin B and G are definitely ruled out as a limiting factor in this dietary deficiency.

The author wishes to express her deep gratitude to Professor U. Suzuki

for his many helpful suggestions throughout this work and to Dr. W. Nakahara for his invaluable advice on the anatomical examination. She is also indebted to the Keimei Society for financial assistance.

Sterilising Action of Acids on Putrifactive Bacteria, Bac. typhosus and Vib. cholerae.

First Report.—Sterilising action of Mineral Acids.

Sogo TETSUMOTO.

(Received September 19, 1932.)

Introduction.

If we compare sterilising power of the same concentration of reagents, we know that acids have stronger sterilising power than bases or salts generally [1] [11]. Owing to this fact, there are many studies about sterilising action of acids.

But before Krönig und Paul's foundamental studies about the sterilising action of reagents, many studies seem the observation of acid molecules only. Krönig and Paul said that P_H are the essential part about sterilising action of acids and that anions and undissociated molecules have very weak or no sterilising power. After this study Bail's, Paul and Birstein's, and many other studies published one after another about this problem.

If we summarise all these studies untill now, are as follows:

(1) Sterilising action of acids depends on P_H almost totally.

(2) Anions or undissociated molecules have no sterilising power or have very weak sterilising power.

But according to my many experiments, of course P_H is the essential part about the sterilising action of acids, following questions arouse;

(1) Are anions and undissociated molecules really weak, or almost no sterilising power?

(2) How are the sterilising power of each reagent under the important condition of the experiment e. g. (to experience always about certain quantities of the microorganisms having constant resisting power)? This condition is very important and that this has been treated carelessly by many experimenters.

(3) How are the collation of sterilising action at the same P_H and at the same normal solution?

(4) Possibility to find out harmless and effective sterilising chemicals and their utilization for drink and diet.

To study and clear up more thoroughly on these questions I performed following experiments. I wish to express my profound thanks to Dr. S. Kojima for his kind leading about my study.

Experiment.

(1) Inoculation of microorganisms.

Taking a sufficient quantity of microorganisms to ascertain their life and death, and then to cause the loss of the toxic action of reagent upon bouillon, I inoculated one platinum loopful of culture: the diameter of the loop was 4 mm., having the diameter of platinum wire 0.6 mm.

(2) Mineral acids used.

HNO_3, HCl, H_2SO_4, H_3PO_4 (ortho), Cromic asid (anhydride), Osmic acid (Osmium tetra oxide), HCNO, H_3BO_3.

(3) Species of microorganisms used for the experiments.

Name	gram	Mobility
Staphylococcus pyogenes aureus	+	−
Bacillus typhosus	−	+
Proteus vulgaris Hauser	+	+
Vibrio cholerae	−	+

(4) Standard culture media.

For the purpose to keep the constant vital forces and resisting power for Staph. c. pyog. aur, Bac. typhosus, Prot. vulgaris, I used next bouillon and agar.

Standard Bouillon: Liebig's meat extract 5g.
Peptone 10″
NaCl 5″
H_2O 1000c.c.
} by NaOH a. q. adjusting to PH 7.0

Standard agar: With above bouillon made 2.5% agar slant.

For Vib. cholerae,

Standard Bouillon: Liebig's meat extract 5g.
Peptone 10″
NaCl 5″
H_2O 1000c.c.
} by 10% Na_2CO_3 adjusting to PH 8.0

Standard agar: With above bouillon made 2.5% agar slant.

(5) Standard resisting power of microorganisms used for experiments.

For 5 days, I repeated 24 hours bouillon cultures of Staph. c. pyog. aureus, Proteus vulgaris H., Bac. tyhosus and Vib. cholerae with standard bouillon, and fixed their respective vital forces. With pure phenol crystal

(M. P. 40°C) I made watery solution of 1/75, 1/90, 1/100, 1/175 dilution by weight.

According to G. Reddish's method[13], I chose next group among many cultivated microorganisms.

Phenol solution

Surviving time (minute)	1/75 dilution	1/90 dilution	1/100 dilution	1/175 dilution
	Staph. coc. pyog.	Bac. typhos.	Prot. vulg.	Vib. chol.
5	+	+	+	+
10	+	±	±	±
15	−	−	−	−

+ ····· alive − ····· death ± ····· alive or death,

With these 4 species I made standard agar slant culture every day at 37°C.

(6) Time.

From the put instant of the inoculation into bouillon, I set the reaction time as follows:

1 m., 2.5, 5, 10, 15, 20, 30, 45, 60, 90, 2 h, 3, 6, 9, 12, 24, 36, e. t. c.
m·····minute h·····hour

New Process Tried On Chemical Sterilisation.

G. Reddish's method used for the chemical sterilisation, is as follows:

Reagent 10 c.c. + 24 hours bouillon culture 1 c.c. of microorganism. This process is used generally now. But if we put 1 c.c. of bouillon culture into 10 c.c. of reagent, concentration and chemical characters of reagent will be greatly changed by it. Above all this fact is distinct when reagent is acid solution. For example;

Reagent	Normal	PH	PH after adding 1 c.c. of Bac. typhos. bouillon culture	PH after adding 1 c.c. of Vib. chol. bouillon culture	PH after adding 0.1 c.c. of Bac. typh. bouillon culture	PH after adding 0.1 c.c. of Vib. chol. bouillon culture
HNO_3	N/100	2.0	2.8—3.0	3.2—3.4	2.1—2.2	2.2—2.4
HCl	〃	〃	〃	〃	〃	〃
H_2SO_4	〃	〃	〃	〃	〃	〃
H_3PO_4	〃	2.1	〃	〃	〃	〃
H_2CrO_4	〃	4.4	5.4—5.6	5.8—6.0	4.6—4.8	4.8—5·0
H_2OsO_4	1/200,000	5.6	6.4—6.6	6.6—6.8	5.8	5.8—6.0
H_3BO_3	1 Nor.	4.8	5.6—5.8	6.0—6.2	5.0—5.2	5.2—5.4
HNO_3	1/1,000	3.0	4.0	4.2—4.4	3.2	3.2—3.4
HCl	〃	〃	〃	〃	〃	〃
H_2SO_4	〃	〃	〃	〃	〃	〃

H_3PO_4	〃	3.2	4.4—4.6	4.6	3.6	3.6
H_2CrO_4	〃	4.8	5.8—6.0	6.0	5.0—5.2	5.0—5.4
H_2OsO_4	1/1,000,000	5.6	6.8—7.0	6.8—7.2	5.8	5.8—6.2

(20°C)

Namely if we put 1 c.c. of bouillon culture of Bac. typhosus into 10 c.c. of reagent, P_H of reagent change 0.8—1.2. On Staph. c. pyog. and Proteus vulgar., we see also nearly the same results. On vib. choler,, the change of P_H is 1.2—1.6. Even if we put 0.1 c.c. of bouillon culture of microorganism, into 10 c.c. of reagent, the change of P_H is 0.1—0.4 on Bac. typhosus, Prot. vulgaris and Staph. c. pyogenes, and 0.2—0.6 on Vib. cholerae. According to this respect Shimokawa[1], Tsubouchi[5] took such process that instead of bouillon culture 1 c.c. they attached microorganism to the silk thread or artificial wild-cocoon silk thread with certain mass and diameter, and put it into reagent. By this method change of reagent will be relatively small. But into such solution as pure dilute acid, silk thread or artificial wild cocoon silk thread will give some change on chemical character of reagent during relatively long time. Krönig and Paul[8] adhered suspension of microorganisms to garnets having the certain size, and put these into reagents. By above methods microorganisms will be much near the silk thread or artificial wild-cocoon thread or garnet and will be not homogeneously distribut in reagents.

To avoid these defects, I tried new process; that is as follows:

I put 0.1 c.c. of bacterial suspension to 10 c.c. of reagent with the pippet measuring 1/100 c.c. degree, to forbid nothing to enter into the reagent except bacterial suspension, and to avoid the change of chemical characters or concentration of reagent.

Bacterial suspension:—

Put 2 mg. of colony of 24 hours standard agar slant culture of microorganism into 10 c.c. of the sterilised physiological NaCl solution and make homogeneous suspension.

By this method nothing can enter into reagents except the microorganism, and that total germs of 0.1 c.c. of bacterial suspension are as follows:

Bac. typhosus Vib. cholerae	20,000,000 germs
Proteus vulgaris Hauser	23,000,000 〃
Staph. c. pyogen. aureus	28,000,000 〃

The change of P_H are as follows:

Reagent	Concentration (Normal)	PH	PH after adding 1 c.c. of Bac. typh. suspension	PH after adding 1 c.c. of Vib. chol. suspension	PH after adding 0.1 c.c. of Bac. typh. suspension	PH after adding 0.1 c.c. of Vib. choler. suspention
HNO_3	1/100	2.0	2.2	2.2—2.4	2.0	2.0
HCl	〃	〃	〃	〃	〃	〃
H_2SO_4	〃	〃	〃	〃	〃	〃
H_3PO_4	〃	2.1	2.4	2.4	2.1	2.1
H_2CrO_4	〃	4.4	4.6	4.6—5.0	4.4	4.4
H_2OsO_4	1/200,000	5.6	5.8	5.8—6.0	5.6	5.6
H_3BO_3	1	4.8	5.0	5.0—5.2	4.8	4.8
HNO_3	1/1,000	3.0	3.2	3.2—3.4	3.0	3.0
HCl	〃	〃	〃	〃	〃	〃
H_2SO_4	〃	〃	〃	〃	〃	〃
H_3PO_4	〃	3.2	3.4—3.6	3.4—3.6	3.2	3.2
H_2CrO_4	〃	4.8	5.0—5.2	5.0—5.4	4.8	4.8
H_2OsO_4	1/1,000,000	5.6	5.8—6.0	5.8—6.2	5.6	5.6

Namely if I put 0.1 c.c. of suspension of Bac. typhosus, or Vib. cholerae, into 10 c.c. of reagent, there is almost no change about PH of the reagent. On Staph. c. pyogenes and Proteus vulgaris Hauser, I saw the same result.

Performance of the experiment: —

Take 2 mg. from each colonies of 24 hours standard agar slant culture of Staph. c. pyog. aureus, Proteus vulgaris Hauser, Bac. typhosus and Vib. cholerae, and put each 2 mg. into 10 c.c. of sterilising physiological NaCl solution and make 4 microorganic suspensions. Make these 4 species of suspensions at 20°C in incubator (difference within 0.5°C). Also keep each series of 10 c.c. taken from reagents at 20°C. Put 0.1 c.c. of the suspension into 10 c.c. of reagent and mixed homogeneously. At the every certain time I inoculate microorganisms from reagents into standard bouillon with the certain platinum loop and cultivate them 48—72 hours in incubator at 37°C. Alive or death of microorganisms are determined by the turbidity of standard bouillon culture. When the result is doubtful 1 repeat the experiment many times, and then I ascertained by morphological investigations and the agglutination test with rabbit's immune serum of each microorganisms.

Results are following tables:

Experimental results: —

Table No. 1. Weight% and PH at *N*/100, *N*/1000, of HNO_3, HCl, H_2SO_4, H_3PO_4 (ortho), Cromic acid, HCNO, 1 Nor. of H_3BO_3, *N*/100,000, *N*/1,000,000 of Osmic acid.

Table No. 2. 3. Sterilising action at *N*/100, *N*/1000, except boric acid. Boric acid……1 Nor.

Table No. 4. Sterilising action of dilute cromic acid and extremly dilute solution e. g. *N*/100,000, *N*/1,000,000 of osmic acid.

Table No. 5. Relation between P_H and normal concentration of mineral acids which affect on sterilisation.

Table No. 6. Sterilising action of anion of each mineral acids.

To ascertain the sterilising action of anion of mineral acids, 1 made alkaline salts having the same anion of each acids. For Staph. coc. pyogen, Prot. vulgar. and Bac. typhos. *N*/100 of alkaline salts of each acids and *N*/100,000 of alkaline osmium salt, 1 nor. of alkaline boric acid salt, are used. For Vib. choler. *N*/1,000 of each alkaline salts, *N*/1,000,000 of alkaline salt of osmic acid, *N*/5,000 of alkaline salt of cromic acid, 1 nor. of alkaline salt of boric acid are used. Each of K and Na salts give nearly the same results, so 1 denote the results of K-salt only.

Table No. 1. Weight % and P_H of reagents.

Reagents	Molecul. weight	Concent. (Normal)	P_H	Weight %	Concent. (Normal)	P_H	Weight %
HNO_3	63.018	1/100	2.0	0.063	1/1,000	3.0	0.0063
HCl	36.468	〃	〃	0.037	〃	〃	0.0037
H_2SO_4	98.016	〃	〃	0.049	〃	〃	0.0049
H_3PO_4	98.064	〃	2.14	0.033	〃	3.2	0.0033
CrO_3	100.01	〃	4.4	0.050	〃	4.8	0.0050
OsO_4	255.0	1/100,000	5.4	0.000128	1/1,000,000	5.6	0.000013
HCNO	43.021	1/100	4.4	0.043	1/1,000	4.6	0.0043
H_3BO_3	61.924	1 Nor.	4.8	2.064			

Table No. 2. Sterilising action at *N*/100 solution.

Name of microorg.	Staph. c. pyog.						Prot. vulgar.						Bac. typhos.						Vib. choler.					
Surviving period	15	20	30	45	60	m 90	1	2.5	5	10	15	m 20	2.5	5	10	15	20	m 30	1	2.5	5	20	30	m 45
HNO_3	+	+	+	−	−	−	+	+	+	±	−	−	+	+	+	±	−	−	−	−	−	−	−	−
HCl	+	+	+	−	−	−	+	+	+	±	−	−	+	+	+	±	−	−	−	−	−	−	−	−
H_2SO_4	+	+	+	−	−	−	+	+	+	±	−	−	+	+	+	±	−	−	−	−	−	−	−	−
H_3PO_4	+	+	+	±	−	−	+	+	+	+	−	−	+	+	+	+	+	−	±	−	−	−	−	−
H_2CrO_4	+	+	−	−	−	−	+	±	−	−	−	−	+	±	−	−	−	−	−	−	−	−	−	−
H_2OsO_4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
HCNO	+	+	−	−	−	−	+	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−
H_3BO_3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	−
Control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

H_3BO_3……1 Normal m……minute +……alive −……death ±……alive or death

Table No. 3. Sterilising action at $N/1000$ solution.

	Staph. c. pyog.						Prot. vulgar.						Bac. typhos.						Vib. choler.					
Surviving period	3	6	9	12	24	36 h	1	2	3	6	9	12 h	2	3	6	9	12	24 h	2.5	5	10	15	20	30 m
HNO_3	+	+	+	±	−	−	+	+	+	−	−	−	+	+	+	−	−	−	+	+	+	±	−	−
HCl	+	+	+	±	−	−	+	+	+	−	−	−	+	+	+	−	−	−	+	+	+	±	−	−
H_2SO_4	+	+	+	±	−	−	+	+	+	−	−	−	+	+	+	−	−	−	+	+	+	±	−	−
H_3PO_4	+	+	+	+	±	−	+	+	+	+	−	−	+	+	+	+	−	−	+	+	+	+	+	−
H_2CrO_4	+	+	±	−	−	−	+	+	−	−	−	−	+	±	−	−	−	−	−	−	−	−	−	−
H_2OsO_4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
HCNO	+	+	−	−	−	−	+	+	−	−	−	−	+	±	−	−	−	−	±	−	−	−	−	−
H_3BO_3 (1 Normal)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±
Control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table No. 4. Sterilising action of dilute Cromic acid and extremely dilute solution of Osmic acid.

	Conc. (Normal)	PH	Staph. c. pyog.					Prot. vulg.				Bac. typhos.				Vib. choler.				
			2.5	5	10	15	20 m	1	2.5	5	10 m	2.5	5	10	15 m	10	15	20	30	45 m
H_2CrO_4	1/2,000	5.2														+	−	−	−	−
"	1/5,000	5.4														+	+	+	+	
H_2OsO_4	1/100,000	5.4	+	+	+	−	−	±	−	−	−	+	+	−	−	−	−	−	−	−
"	1/200,000	5.6	+	+	+	+	−	+	+	−	−	+	+	±	−	−	−	−	−	−
"	1/500,000	"														−	−	−	−	−
"	1/1,000,000	"														+	−	−	−	−
Control			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table No. 5. Sterilising action of the same PH of mineral acids, such as HNO_3, HCl, H_2SO_4, H_3PO_4.

PH 2.0	Staph. c. pyogen.				prot. vulgar.				Bac. typhos.				Vib. Choler.			
	15	20	30	45 m	2.5	5	10	15 m	5	10	15	20 m	1	2.5	5	10 m
HNO_3	+	+	+	−	+	+	±	−	+	+	±	−	−	−	−	−
HCl	+	+	+	−	+	+	±	−	+	+	±	−	−	−	−	−
H_2SO_4	+	+	+	−	+	+	±	−	+	+	±	−	−	−	−	−
H_3PO_4	+	+	+	−	+	+	±	−	+	+	±	−	−	−	−	−

PH 3.0	Staph. c. pyogen.				Prot. vulgar.				Bac. typhos.				Vib. choler.			
	6 h	9 h	12 h	24 h	2 h	3 h	6 h	9 h	3 h	6 h	9 h	12 h	10 m	15 m	20 m	30 m
HNO_3	+	+	±	−	+	+	−	−	+	+	−	−	+	±	−	−
HCl	+	+	±	−	+	+	−	−	+	+	−	−	+	±	−	−
H_2SO_4	+	+	±	−	+	+	−	−	+	+	−	−	+	±	−	−
H_3PO_4	+	+	±	−	+	+	−	−	+	+	−	−	+	±	−	−

Table No. 6. Sterilising action of anions of mineral acids.

	Staph. c. pyog.					Prot. vulgar.						Bac. typhos.						Vib. choler.						
Surviving period	m 10	15	m 20	d 6	7	d 8	2.5	5	m 10	d 4	5	d 6	m 10	15	m 20	d 5	6	d 7	m 10	15	20	m 30	60	h 6	9	12
KNO_3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
KCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
K_2SO_4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
K_3PO_4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
K_2CrO_4	+	+	+	+	±	−	+	+	+	±	−	−	+	+	+	+	±	−	+	+	+	+	±	−	−	−
K_2OsO_4	+	+	−	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	+	+	−	−	−	−	−	−
KCNO	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
K_3BO_3	+	+	+	+	±	+	+	+	+	±	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−
Control	+	+	+	±	−	−	+	+	+	±	−	−	+	+	+	+	±	−	+	+	+	+	+	+	+	±

m……minute h……hour d……day.

Summary.

(1) Sterilising action of mineral acids such as HNO_3, HCl, H_3PO_4 is determined by PH of each solution, and has no relation about kind of acids.

(2) Anions of strong mineral acids have no sterilising action.

(3) There is no special relation between the value of molecular weight of mineral acids and sterilising action.

(4) On sterilising action of cromic acid and HCNO, PH concerns a little.

(5) Sterilising action of dilute cromic acid and HCNO, such as $N/100$ are almost due to the action of undissociated acid molecule.

(6) The violent sterilising action of Osmic acid is chiefly due to the action of its anion and PH concerns a little.

(7) Sterilising action of Boric acid is chiefly due to PH and the strilising action of it is very feeble.

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